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Phytochemical Study and Evaluation of the Antibacterial Activity of *Acacia Amythethophylla* Steud ex a. Rich Extracts. (Fabaceae) in the Treatment of Urinary Infection in Chad

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Abstract

Urinary infections are a public health problem worldwide and in Chad. To reduce these problems, plants were used for traditional treatment. The objective of this study was to reconcile modern medicine and ethnomedicine by testing Acacia amythethophylla extracts and antibiotics commonly used against urinary infections in our country. In this study, a phytochemical screening was carried out on the bark of the trunk of Acacia amythethophylla. The isolation and identification of bacteria responsible for urinary infections were carried out using standard clinical microbiology methods.

The yield rates of our hydroethanolic and aqueous extracts from Dourbali and Pala were 17.7%, 26.3%; 8.7% and 10.5% respectively. Phytochemical analysis revealed the presence of flavonoids, tannins, saponosides and alkaloids whose antibacterial activity is confirmed. Additionally, Acacia amythethophylla extracts inhibited the growth of bacteria at MICs of 1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL. Standard techniques made it possible to determine the prevalence of bacteria responsible for urinary infections: Escherichia coli (39.13%), Staphylococcus (17.39%) and Pseudomonas aeruginosa (8.69%). The antibiogram revealed varied resistance of bacteria to aminopenicillins, on the other hand, resistance of Staphylococcus to methicillin and 100% sensitivity to fusidic acid.

This study not only revealed the high resistance rate of the bacteria most frequently involved in urinary infections, but also showed that the hydroethanolic and aqueous extracts of the bark of Acacia amythethophylla could be a potential supplier of secondary metabolites with antibacterial activities, allowing the development of a new molecule to fight against urinary infections.

***Keywords**: Acacia amythethophylla, Antibacterial activity, Antibiotic, Urinary tract infection, Chad.*

Introduction

Urinary tract infection (UI) is defined as an attack on all or part of the urinary tract by one or more microorganisms which generate an inflammatory reaction and clinical manifestations [1]. Caused mainly by bacteria, urinary infections constitute a public health problem representing throughout the world one of the main reasons for consultation and intensive use of antibiotics, resulting in the appearance of multi-resistant strains both in hospital environment than in the community environment [2]. Around 150 million cases of urinary tract infection worldwide and two (2) million in France are recorded per year [3]. The prevalence of urinary infections is more often high in women than in men [4].

The management of these infections requires the prescription of antibiotics which has today become a problem due to the emergence of multi-resistant bacteria in the human population. The occurrence of this antibiotic resistance is linked on the one hand to the irrational use of antibiotics, and is the main cause of therapeutic failures [5, 6]. These infections are mainly caused by enterobacteria, the most cited of which is Escherichia coli in 70 to 80% of cases [7, 8, 9, 10].

In Chad, work has shown high prevalence of resistance of these enterobacteria to commonly used antibiotics. Bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* are known for theirresistance to antimicrobials [11, 12, 13].

The search for a therapeutic alternative for better care is essential. One of the strategies for this research consists of exploring plants used in traditional medicine which 80% of the world population, and in particular Africa, have used, given its accessibility and affordable cost [14, 15]. The plants most commonly used in the treatment of urinary infections are *Vaccinium microcarpum*, etc. Among the most used plants is *Acacia amythethophylla*. Thespecies of this family are harvested for human and animal food purposes, as well as for their oil,

their fibers, as fuel, their wood, and their use in medicine and chemistry [16, 17, 18]. The progression of multi-resistance and the absence of real prospects for the discovery of new antibiotic molecules in the years to come, led us to study the effectiveness of *Acacia amythethophylla* in order to identify or reveal the active ingredients with active antibacterial. The objective of this work was to evaluate the antibacterial activity of *Acacia amythethophylla* extracts on the bacteria responsible for urinary infections.

The present study made it possible to highlight the richness of the hydroethanolic and aqueous extracts of the bark of the trunk of *Acacia amythethophylla* in compounds that could be a potential source of natural biomolecules to fight against bacterial infections.

Material and methods

Setting, Type and period of study

The town of Pala, the town of Dourbali where the harvest of the trunk bark of *Acacia amythethophylla* was carried out.

The Laboratory Bacteriology Unit of the National Reference University Hospital Center of N'Djamena (CHU- RN) served as a framework for isolating the bacterial strains responsible for urinary infection.

The Bacteriology Unit of the Diagnostic Research and Scientific Expertise Laboratory (LaboReDES) of the Faculty of Human Health Sciences (FSSH), University of N'Djamena served as a framework for the evaluation of bacterial activity with hydroethanolic and aqueous extracts of *Acacia amythethophylla*.

This was a prospective and experimental study that lasted twelve months (12 months), running from February 2022 to January 2023.

Biological material

The biological material consisted of the bark of the trunk of *Acacia amythethophylla* and bacteria isolated from urine samples. Antimicrobial tests were carried out with 4 bacterial strains: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* and *Staphylococcus aureus* isolated from clique samples in patients suffering from urinary infections in the urology department of the National Reference University Hospital Center of N'Djamena (CHU-RN)

Quality control was carried out using the reference strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028 provided by the CHU-RN.

Sample collection

Isolation and identification of bacteria

Cystine lactose electrolyte deficient (CLED) and Chapman agar plates were used to isolate bacteria; Catalase, coagulase and API20E galleries served as biochemical identification tests. Mueller-Hinton (MH) agar was used to purify bacterial strains and at the same time served for antibiotic susceptibility testing and evaluation of bacterialactivity of hydroethanolic extracts of tree trunk bark extracts. *Acacia amythethophylla*.

Technique for collecting plant samples

The collection of samples was carried out thanks to the support of certain traditional practitioners present in the harvesting areas. The identification and confirmation of the species was made at the herbarium of the Institute of Research for Development (IRED).

Pharmacological and biological study

Extractions

The preparation of the extracts was done at LaboReDES in the pharmacology unit. The barks were cut into small pieces and dried away from the sun, at laboratory temperature for about three weeks. Once dried, they were reduced to a fine powder using a grinder. This powder was used to prepare the different extracts.

Determination of yield

The different extracts obtained were weighed and the yield was calculated.R (%)= M/M0×100

M: Mass in grams of the resulting dry extract after evaporation.

M0: Mass in grams of dry powdered plant material.

A: yield

Phytochemical screening of secondary metabolites

The aim of this study was to research the different chemical groups contained in plant extracts that could be responsible for the antibacterial activity.

Principle: this involves carrying out a chemical screening on the organs (barks) of the plant. The phytochemical screening was carried out according to the standard method adapted by the chemistry laboratory of the Faculty of Exact and Applied Sciences (FSEA) of the University of N'Djamena.

Protocol: the plant material collected and dried away from the sun, crushed, pulverized and stored in a clean anddry container was used to carry out the identification test according to standard methods of secondary metabolites adapted by Mamout [19].

We prepared a 5% infusion.

Procedure: we weighed 0.5 g of the powder, boiled the water, took 10 mL of the boiling water and mixed with the powder and left to cool for a few minutes, the solution obtained is a 5% infusion.

Carrying out the test: Action of FeCl3: we added a few drops of FeCl3 to the infusion. Coloring with precipitate indicates the presence of tannins:

- ✓ Blue-black coloring: gallic tannin;
- ✓ Brown-green coloring: catechic tannins.
- ❖ Flavonoids.

The test is carried out on infusing at 10%.

Procedure: we weighed 5 g of the vegetable powder to which we added 50 mL of boiling water then left it to infuse for a few minutes and filtered.

Carrying out the test: we took 3 mL of the filtrate then added 3 mL of the HCL-CH3OH-H2O mixture in an equal volume (1/1/1) and some magnesium shavings. The presence of flavonoids in the solution is manifested bycolorations:

- ✓ In orange: these are flavones;
- ✓ In red: these are flavonols;
- ✓ In purple: these are flavonones.
- ❖ Sterols and terpenoids

The test is carried out on the ethereal extract of plant material

Procedure: we macerate 1 g of the vegetable powder in 20 mL of ether for 24 hours in closed bottles then filtered.

Carrying out the test:
LIBERMAN test: we evaporated a few drops of the ethereal solution on a watch glass. The residue is dissolved in two (2) drops of acetic anhydride, to which one drop of concentrated sulfuric acid is added. The presence of sterols or terpenoids is highlighted by the purple coloring which turns green.
SALKOWSKI test: we added chloroform and concentrated sulfuric acid to the ethereal residue and then stirred. The presence of sterols or terpenoids is characterized by the formation of layers, the upper layer becomes red andthe lower layer becomes yellow.

❖ **Alkaloid**

Procedure: we weighed 0.5 g of the vegetable powder to which 15 mL of 95° ethanol was added. After stirring for 30 minutes, the extract was filtered.

Carrying out the test: 5 mL of the extract is taken, 3 mL of 1 N HCl and a few drops of the following reagent are added: **MAYER, DRAGGENDORFF or WAGNER**. The test is positive if there is formation of a strong precipitate in suspension or immediate flocculation.

❖ **Free quinones**

For the search for free quinones, we weighed 1 g of plant material and placed it in 20 mL of petroleum ether. After stirring and resting for 24 hours, the extract is filtered and concentrated. The presence of free quinones is confirmed by the addition of a few ml of 1/10 NaOH, when the aqueous phase turns yellow, red or purple.

❖ **Saponosides**

The principle consists of determining the persistent foam index for a period of 30 minutes of a 2% aqueous decoction. After cooling and filtration, the volume is readjusted to 100 mL with demineralized water.
In a series of 10 test tubes numbered 1 to 10, successively introduce 1, 2, 3.10 mL of decoction. Adjust the volume of each tube to 10 mL with distilled water. Each tube is shaken horizontally for 15 seconds. After resting for 15 minutes in a vertical position, the height of the persistent foam is measured in cm. The dilution in the tube or the height of the foam is equal to 1 cm represents the desired index. If it is close to 1 cm represented in the Xth tube, we calculate the foam index by the following formula:
I= (5×height of foam in cm in the Xth tube × 100) /x.With X=1, 2, 3…10.
The presence of saponin in the plant is confirmed with an index greater than 100 cm

❖ **Anthraquinone**

In a test tube we moistened 2 g of vegetable powder with 2 mL of a 10% HCL solution. Add 20 mL of chloroform and leave to macerate for 24 hours. Filter. Take 1 mL of the chloroform solution and treat it with 1 mL of 10% NaOH. A red color indicates the presence of quinones.

❖ **Anthocyanin**

Test carried out on the 5% infusion (0.5 g of plant material is placed in 10 mL of boiling water for 15 minutes and filtered. We carried out the hydrolysis with 3 mL of alcoholic HCL (HCL + ethanol v/v) on the infused by heating for a few minutes at 50°C in a Marie bath. The formation of the purplish red color indicates the presence of anthocyanins.

❖ **Cardiotonic glycoside**

1 g of plant material is macerated in 10 mL of chloroform for 15 min. after filtration, 4 mL of extract is placed ina test tube; 2 mL of acetic anhydride are added and left to cool for a few minutes in an ice bath. 1 mL of concentrated H2SO4 is slowly added against the wall of the tube. The change in color from purple to blue or green indicates the presence of the aglycone of cardiotonic glucosides.

The results are classified according to:
- ✓ Frankly positive reaction (+++);
- ✓ Moderately positive reaction (++);
- ✓ Weakly positive reaction (+);
- ✓ Negative test (-).

Quantitative assessment of total polyphenol content

Polyphenols are commonly subdivided into flavonoids (flavones, flavonols, anthocyanidins, isoflavones, flavonones, catechins) or non-flavonoids (resveratrol, phenolic acids, lignans). Polyphenols are powerful antioxidants that can help neutralize free radicals.
The determination of total polyphenols was carried out according to the method described by (Singleton et al., 1999) taken up by (Karou, 2006) [20]. It evaluates all reducing phenolic compounds from the Folin-Ciocalteu reagent (FCR) consisting of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMO12O40). It is a redox reaction in a basic medium during which the OH function of the phenols is oxidized while the RCF is reduced to a mixture of blue oxides of tungsten (W8O23) and molybdenum (MO8O23) with formation of a phenolate ion.

Quantitative assessment of proanthocyanidol content

Polyphenols are commonly subdivided into flavonoids (flavones, flavonols, anthocyanidins, isoflavones, flavonones, catechins) or non-flavonoids (resveratrol, phenolic acids, lignans). Polyphenols are powerful antioxidants that can help neutralize free radicals.
The proanthocyanidol content was evaluated by the Butanol-HCl method, described by (Porter et al., 1985) [21]. The method is based on the oxidative depolymerization reaction of condensed tannins in an acidic medium (catalyst). The reaction leads to the release of anthocyanidins (colored molecules) corresponding to the cleaved monomers which absorb at 540 nm.

Preparation of tested solutions

The solvents used consisted of distilled water for the aqueous extract, and DMSO (dimethyl sulfoxide) for the hydroethanolic extracts. Thus 200 mg dissolved in 10 mL or (20 mg/mL).

Determination of the minimum inhibitory concentration (MIC).

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

Sterility tests

The extracts are sterilized by filtration on a 0.45 nm millipore membrane.
We spread 100 µL of each extract on MH. Extracts that have not shown growth are considered pure.

Preparation of dilutions

A concentration range was prepared from the crude aqueous extract with a concentration of 20 mg/mL using the two-in-two dilution method (1/2, ¼, 1/8, 1/16, 1/32), in the five test tubes that we added to the first tube (1/2), 10mL of the crude aqueous extract, then homogenized to obtain a 1/2 dilution solution, then from the latter we took5 mL that we introduced into the second tube (1/4) added 5mL then homogenized to obtain a 1/4 dilution solution. And the same steps were repeated until the last solution at 1/32 dilution was obtained. The same operation carried out for the hydroethanolic extract.

Determination of antibacterial activityPreparing the discs

The method used was the method of Kirby and Bauer (1982) [22]. It is based on the diffusion of paper discs impregnated with aqueous and hydroethanolic extract of *Acacia amythethophylla* on Mueller-Hinton (MH) agar. Discs not loaded with antibiotics with a diameter of 6 mm, sterilized then impregnated with 20 µL of the aqueous and hydroethanolic solution of *Acacia amythethophylla* at increasing concentrations of 1.25 mg/mL, 2.5 mg/mL, 5 mg /mL, 10 mg/mL and 20 mg/mL. The inoculum used was 10^8 CFU/mL. Positive control disks (discs loaded with extracts) and negative control disks (disks not loaded with extracts). Ciprofloxacin was used as the reference antibiotic. Finally, the disks were incubated at 37° C for 24 h.

The diffusion method is carried out as follows:

- ✓ **Preparation of the inoculum**: From a pure and fresh culture we prepared a suspension with an opacity equivalent to the 0.5 Mac Farland standard.
- ✓ **Inoculation of the boxes**: By flooding, the inoculum was inoculated into the Mueller Hinton agar.
- ✓ **Arrangements of the discs loaded with aqueous and hydroethanolic extracts of *Acacia amythethophylla***: the discs were placed on the MH agar using a dispenser or with forceps by pressing them lightly, and were placed at a minimum of 15 mm from the periphery of the box so that the inhibition zones do not overlap. A concentration gradient of the plant extract is thus formed around each disk;
- ✓ **Incubation**: Incubate the boxes at 37 ° C for 24 hours;
- ✓ **Reading the diameter of the inhibition zones**: No growth appears when the plant extract is present at the minimum inhibitory concentration and is sensitive to the strain. It is then possible to measure, using a caliper,the diameter of the inhibition zone which is directly proportional to the minimum inhibitory concentrations.
- ✓ **Interpretation**: After measuring the inhibition zone translated by a clear zone around the aqueous and hydroethanolic extract of the plant, we deduce that the larger the diameter of the zone, the more sensitive the extract of the plant is.

This same method was used to determine the sensitivity of the bacteria to conventional antibiotics.Table 1: Standard used for reading the results of antibiogram tests on plant extracts [23, 24].

Inhibition diameter	Degree of sensitivity of the germ
Ø < 7 mm	Insensitive
7 mm ≤ Ø < 8 mm	Sensitive
8 mm ≤ Ø < 9 mm	Quite sensitive
Ø ≥ 9 mm	Very sensitive

Antibiogram of germs isolated in urine

A bacterial suspension was prepared in sterile distilled water from a 24-h pure culture from nutrient agar. This suspension was compared to the standard of the McFarland 0.5 solution which corresponds to 10^8 CFU/mL. The suspensions thus obtained were inoculated by swabbing onto Mueller Hinton agar. The thickness of (Bonnet et al., 2013) [25]. The antibiotic disks were placed on Petri dishes. After 10 to 15 minutes, the plates were incubated at 37 ° C for 24 hours. The diameters of the inhibition zones were measured and compared to the sensitive ones.

Table 2: guide to interpreting antibiotic inhibition diameters (CA-SFM 2016-2020; NCCLS, 1998) [25, 26].

Antibiotic	Disk Load (mm)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Beta lactams				
Ampicillin (AMP)	10µg	Ø < 15	15-21	Ø ≥ 21
Amoxicillin (AMX)	25µg	Ø < 14	14-21	Ø ≥ 21
Amoxicillin+clavulanic acid (AMC)	20/10µg	Ø < 17	17-22	Ø ≥ 22
Methicillin (MET)	5µg	Ø < 16	16-19	Ø ≥ 19
Ceftriaxone (CRO)	30µg	Ø < 20	20-22	Ø ≥ 23
Ceftazidime (CAZ)	10µg	Ø < 21	21-27	Ø ≥27
Imipenem (IMP)	10µg	Ø < 19	19-21	Ø ≥ 22
Fluroquinolones				
Ciprofloxacin (CIP)	5µg	Ø < 19	19-21	Ø ≥ 22
Aminoglycosides				
Gentamicin (GMN)	10 µg	Ø < 14	14-16	Ø ≥ 16
Fusidic acid				
Fusidic acid (FAD)	10 µg	Ø < 24	24-29	Ø ≥ 29
Glycopeptides				
Vancomycin (VCN)	5 µg	Ø < 17	17-23	Ø ≥ 23

Data analysis

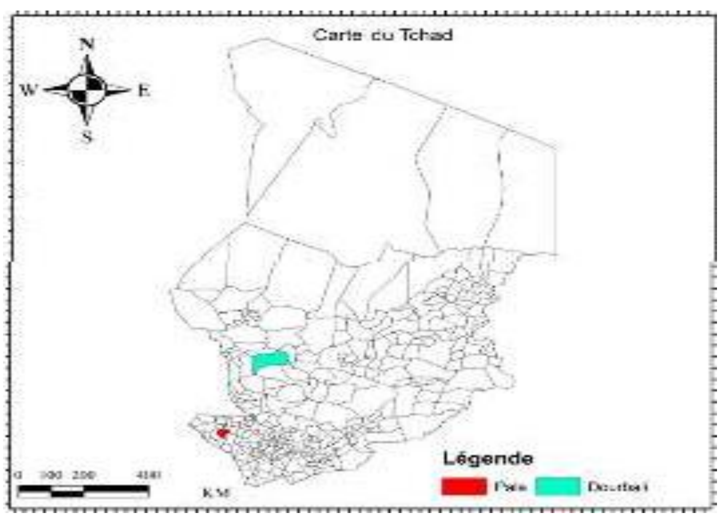
The data were analyzed using Microsoft Excel 2010 software, presented in the form of tables and figures. The report was written using Microsoft Word 2010. Experiments were performed in triplicate and mean values are presented with standard deviation (mean ± SD).

Results

Geographical location of study areas

The bark samples of the plant were collected in Dourbali in the Chari-Baguirmi region and in Pala in western Mayo-Kebbi. The locality of Pala is located approximately 500 km southwest of Chad and borders the Republic of Cameroon. The different parts of the plant were collected from Dourbali, a locality located 100 km east of N'Djamena in theprovince of Chari Baguirmi. Rich in its flora variabilities, these two provinces abound in a significant quantity of*Acacia amythethophylla* used in the treatment of urinary infections.

Figure 1: Presentation of the plant harvest area on the map of Chad.



Extraction yield

The extraction yield of the different extracts (aqueous and hydroethanolic) from the bark of *Acacia amythethophylla* obtained after a test portion of 10 g of each sample is represented in table 1.

The best yield was obtained with the hydroethanolic extract of Pala, 26.3% and Dourbali 17.7%.Table 3: Evaluation of the yield of plant extracts.

yield		Dourbali	Pala
Hydroethanolic	%	17.7	26.3
Aqueous %		8.7	10.5

Phytochemical screening

Highlighting the different classes of secondary metabolites in our extracts allowed us to have an idea of the pharmacological activities. For this we carried out phytochemical tests on the different extracts prepared from the bark of *Acacia amythethophylla* from Pala and Dourbali. The results are summarized in Table 4. At the end of the photochemical analysis, extracts of the barks of *Acacia amythethophyllla* from Dourbali and Pala showed their richness in tannins, alkaloids, saponosides, free quinones, anthocyanins, sterols and terpenoids; cardiotonic glycosides, anthraquinones and flavonoids. Free Anthraquinones and Quinones are absent in the extract of the bark of the Dourbali trunk.

Table 4: Qualitative phytochemical screening of *Acacia amythethophylla* extracts.

Extact	Chemical group								
	Alkaloids	Tannins	Flavonoids	Anthraquinone	Free quinones	Heterosides	Saponosides	Anthocyanins	Sterols and Terpenoids
EaqAaD	++	+	+	-	-	+	+	+	+
EaqAaP	++	++	++	-	-	+	++	+	+
EhydroethAaD	+++	++	+	-	-	+	++	++	++
EhydroethAaP	+++	+++	+++	-	-	++	+++	+++	++

EaqAa D = aqueous extract of *Acacia amythethophylla* from Dourbali; EaqAaP = aqueous extract of *Acacia amythethophylla* from Pala;

EhydroethAaD = hydroethanolic extract of *Acacia amythethophylla* from Dourbali; EhydroethAaP = hydroethanolic extract of *Acacia amythethophylla* from PalaLegend: (-) = Totally absent; (+) = Weakly positive; (++) = Moderately positive; (+++) = Frankly positive.

The results of the quantitative analysis showed the trunk barks of *Acacia amythethophylla* had a higher total polyphenol content (0.78 ± 0.003 mgAGE/g). The hydroethanolic extracts of *Acacia amythethophylla* present the highest values than the aqueous extracts (table 5). The different proanthocyanidol contents in the recipe ranged from 0.20% ± 0.001 to 0.36% ± 0.003 catechin equivalents per gram.

Table 5: Quantitative analysis of extracts.

extracts	Total polyphenols (mgAGE/g)	Proanthocyanidins (mgCE/g)
EaqAaD	0.30±0.008	0.20%±0.001
EaqAaP	0.50±0.006	0.36%±0.003
EhydroethAaD	0.51±0.006	0.21%±0.001
E4hydroethAaP	0.78±0.003	0.32%±0.003

mgAGE/g: milligram equivalent of gallic acid per gram of extract; mgCE/g: milligram of catechin per gram of extract.

Antibacterial activity of extracts

The tables below indicate the inhibition diameter of the aqueous and hydroethanolic extract of the bacterial strains tested at different concentrations (1.25 to 20 mg/ mL).

Minimum inhibitory concentration of Pala aqueous extract

The aqueous extract of the trunk bark of *Acacia amythethophylla* de Pala inhibits the growth of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* at the minimum inhibitory concentration (MIC) of 1.25 mg/Ml (table 6).

Table 6: Minimum inhibitory concentration of Pala aqueous extract

CMI	*K.P*	SU	*P.A*	SU	*E.C*	S	*S.A*	SU
20mg/mL	18.5±0.7	S	10±0.0	S	12±1	S	8.5±0.5	S
10mg/mL	8±0.0	S	8.5±0.6	S	9±0.0	S	9±0.0	S
5 mg/mL	8±.,0	S	8.5±0.6	S	8±0.0	S	8±0.0	S
2,5mg/mL	8±0.0	S	7.5±0.5	S	7.5±0.5	S	8±0.0	S
1.25mg/mL	8±0.0	S	7.5±0.5	S	7±0.0	S	7±0.0	S

MIC = Minimum inhibitory concentration; S= sensitive; K.P: *Klebsiella pneumoniae*; P. A = *Pseudomonas aeruginosa*; SU= susceptibility; E. C = *Escherichia coli*; S.A = *Staphylococcus aureus*

Minimum inhibitory concentration of Dourbali aqueous extract

The aqueous extract of the trunk bark of *Acacia amythethophylla* from Dourbali inhibits the growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* at the minimum inhibitory concentration (MIC) of 1.25 mg/ml. Only *Staphylococcus aureus* is inhibited at the MIC of 5 mg/mL.

The initiation of the growth of *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa i*nduced by the hydroethanolic extract of the bark of the trunk of *Acacia amythethophylla* de Pala at different concentrations is presented in Table 7.

Table 7: Minimum inhibitory concentration of the aqueous extract of Dourbali

CMI	K. P	SU	P. A	SU	E. C	SU	S. A	SU
20 mg/mL	9±1.0	S	9.5±0.5	S	8±0.0	S	9±1	S
10 mg/mL	9±1.0	S	9±0.0	S	8±0.5	S	10±1	S
5 mg/mL	8±0.0	S	8±0.0	S	7.5±0.5	S	8.5±0.5	S
2.5 mg/mL	7.5±0.5	S	7.5±0.5	S	8±1.0	S	6±0.0	I
1.25mg/mL	7.5±0.5	S	7±0.0	S	7.5±0.5	S	6±0.5	I

S= sensitive; K.P: *Klebsiella pneumoniae*; SU= susceptibility; I= Insensitive; P.A = *Pseudomonas aeruginosa*; E. C = *Escherichia coli*; S.A = *Staphylococcus aureus*

Minimum inhibitory concentration of Pala hydroethanolic extract

The hydroethanolic extract of the trunk bark of *Acacia amythethophylla* de Pala inhibits the growth of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* at the minimum inhibitory concentration (MIC) of 1.25 mg/mL

Table 8: Minimum inhibitory concentration of Pala hydroethanolic extract

CMI	K. P	SU	P. A	S	E. C	SU	S. A	SU
20 mg/mL	25±2.0	S	10±0.0	S	25±0.5	S	20±0.0	S
10 mg/mL	20±0.0	S	9±0.0	S	15.5±0.5	S	10.5±0.5	S
5 mg/mL	8±1.0	S	8.5±0.5	S	8.5±0.5	S	10±0.0	S
2.5mg/mL	8±1.1	S	7±0.0	S	8±0.0	S	9±0.0	S
1.25mg/mL	7±0.0	S	7±0.0	S	8±0.0	S	8±0.0	S

S= sensitive; K.P: *Klebsiella pneumoniae*; SU= susceptibility; P.A = *Pseudomonas aeruginosa*; E. C = *Escherichia coli*; S.A = *Staphylococcus aureus*

Minimum inhibitory concentration of Dourbali hydroethanolic extract

The hydroethanolic extract of the bark of the trunk of *Acacia amythethophylla* from Dourbali inhibits the growth of *Staphylococcus aureus*, *Klebsiella pneumoniae and Escherichia coli* at the minimum inhibitory concentration (MIC) of 1.25 mg/mL, on the other hand *Pseudomonas aeruginosa* is inhibited at the MIC of 2.5 mg/mL.

Table 9: Minimum inhibitory concentration of the hydroethanolic extract of Dourbali.

CMI	*K.P*	SU	P.A	SU	*E.C*	SU	*S.A*	SU
20mg/mL	14±2.0	S	11.5±0.5	S	13±1.0	S	13.5±0.5	S
10mg/mL	9±1.0	S	9.5±0.0	S	11±1.0	S	13.5±0.5	S
5mg/mL	12±1.0	S	7±0.5	S	13.5±0.5	S	15±2.0	S
2.5mg/mL	10±0.0	S	7.5±1.0	S	10±0.0	S	9±0.0	S
1.25mg/mL	7.5±0.5	S	6.5±0.5	I	8.5±0.5	S	8±0.0	S

S= sensitive; K.P: *Klebsiella pneumoniae*; SU= susceptibility; I= insensitive; P.A = *Pseudomonas aeruginosa*; E. C = *Escherichia coli*; S.A = *Staphylococcus aureus*

Study of the sensitivity of isolated bacteria to antibiotics

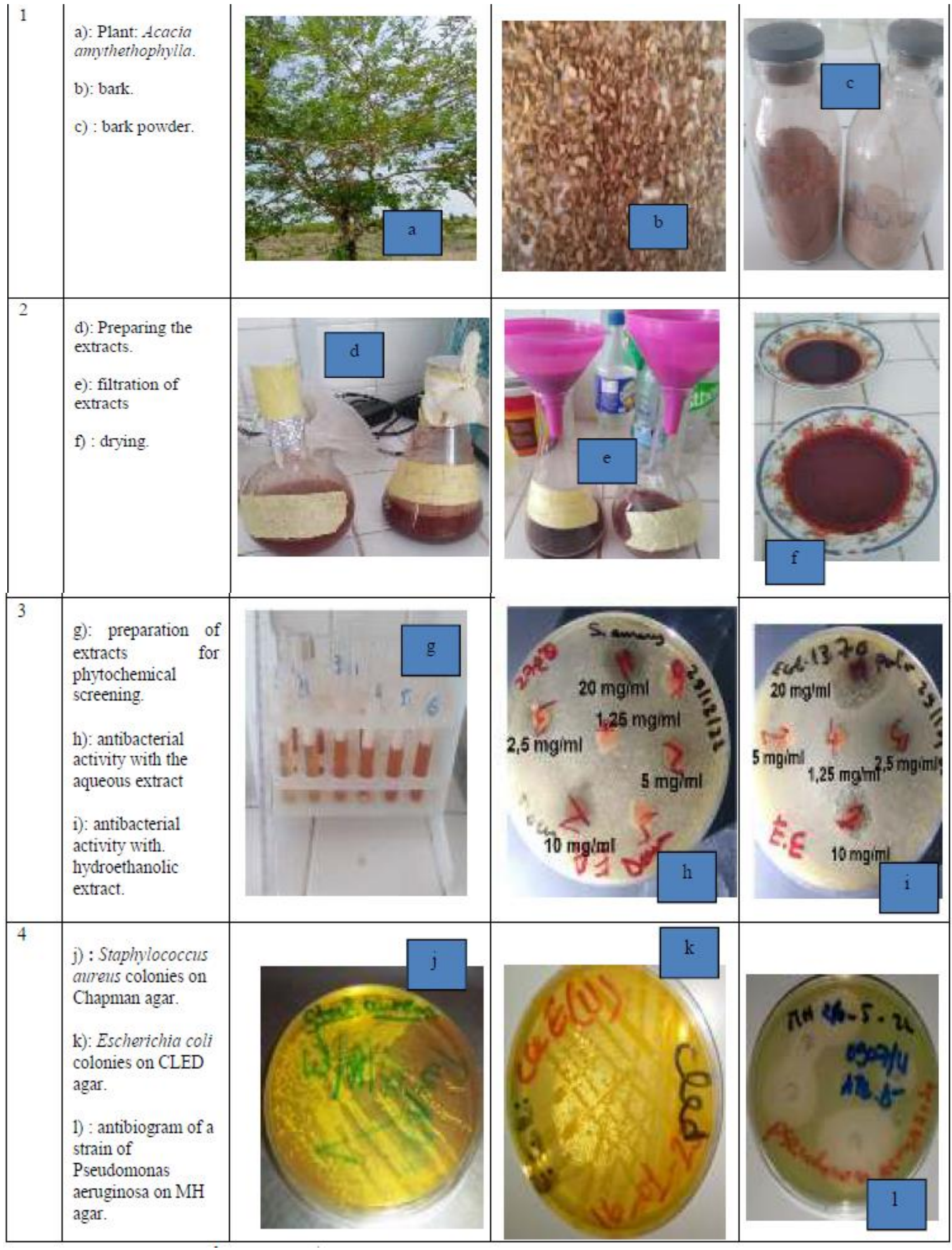
The table below shows the result of antibiotic sensitivity tests from different families. The proportions of resistance are completely variable. *Staphylococcus* are completely (100%) resistant to methicillin and 100% sensitive to fusidic acid. The results obtained revealed fairly high rates of resistance in different bacterial strains isolated to aminopenicillins: ampicillin (69.5%), amoxicillin (56.5%) and amoxicillin + clavulanic acid (35%).

Table 10: Evaluation of the effectiveness of antibiotics

Agents bacterial	Nb	Antibiotic																					
		AMP		AMX		AMC		MET		CRO		IMP		CAZ		GMN		CIP		FAD		VCN	
		R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S	R+I	S
EC	18	14	4	11	7	7	11	NR	NR	12	6	5	13	8	10	7	11	5	13	NR	NR	NR	NR
SA	8	5	3	4	4	2	6	8	0	4	4	2	6	2	6	4	4	2	6	0	8	2	6
SH	4	2	2	1	3	0	4	4	0	1	3	0	4	0	4	1	3	0	4	0	4	2	2
KP	3	2	1	1	2	1	2	NR	NR	1	2	0	3	1	2	1	2	3	0	NR	NR	NR	NR
AB	2	2	0	2	0	2	0	NR	NR	1	1	0	2	0	2	1	1	1	1	NR	NR	NR	NR
EC	2	0	2	1	1	0	2	NR	NR	1	1	0	2	0	2	1	1	0	2	NR	NR	NR	NR
PM	5	4	1	3	2	2	3	NR	NR	2	3	0	5	1	4	3	2	1	4	NR	NR	NR	NR
PA	4	3	1	3	1	2	2	NR	NR	2	2	1	3	1	3	2	2	1	3	NR	NR	NR	NR
Total (%)	46	32 (69.5)	14 (30.4)	26 (56.5)	20 (43.5)	16 (35)	30 (65.2)	12 (100)	0 (0)	24 (52.2)	22 (48)	8 (17.4)	38 (83)	13 (28.3)	33 (72)	20 (43.5)	26 (56.5)	13 (28.3)	33 (72)	0 (0)	12 (100)	4 (33.3)	8 (66.6)

E. C = *Escherichia coli*; K.P = *Klebsiella pneumoniae*; S.A = *Staphylococcus aureus*; SH = *Staphylococcus haemolyticus*; AB=*Acinetobacter baumannii*; EC = *Enterobacter cloacae*; PM = *Proteus mirabilis*; P.A = *Pseudomonas aeruginosa;* Nb = number, NR = not required, AMP = Ampicillin, AMX = Amoxicillin, MET = Methicillin, CAZ = Ceftazidime; GMN = Gentamicin, Cip = Ciprofloxacin, FAD = Fusidic Acid, VCN = Vancomycin, R+I = R: resistant + Intermediate = Resistant; S= sensitive.

Table 11: The biotechnological steps for isolating bacterial strains, collecting and making bark from the trunk of *Acacia amythethophylla.*



1	a): Plant: *Acacia amythethophylla.* b): bark. c) : bark powder.	a	b	c
2	d): Preparing the extracts. e): filtration of extracts f) : drying.	d	e	f
3	g): preparation of extracts for phytochemical screening. h): antibacterial activity with the aqueous extract i): antibacterial activity with. hydroethanolic extract.	g	h	i
4	j) : *Staphylococcus aureus* colonies on Chapman agar. k): *Escherichia coli* colonies on CLED agar. l) : antibiogram of a strain of Pseudomonas aeruginosa on MH agar.	j	k	l

Source: Haoua et *al*., 2022-2023)

Discussion

The study initially consisted of harvesting the bark from the trunk of *Acacia amythethophylla* and characterizing the secondary metabolites they present. After pharmacological analysis, the yield of the extract varied depending on the solvent used. The best yield was obtained with the hydroethanolic extract of the plant harvested in Pala (26.3%) followed by that of Dourbali (17.7%). The yield was low with the aqueous extract, it was 10.5% and 8.7% respectively on the Pala and Dourbali samples.

The hydroethanolic extract (macerate) gives the best extraction yield, which could indicate that 70% ethanol would be the ideal solvent for extracting a large majority of chemical constituents from the plant studied. These results corroborate those of Koudoro et al who reported that the hydroethanolic extract is higher with a percentage of (13.65%) followed by the aqueous extract (8.85%) on the bark of the trunk of *Acacia polyacantha* same genus as*Acacia amythethophylla* during a study carried out in Benin in 2009 [27]. This could be explained by the influence of ecology, Pala is located in the Sudanian zone and Dourbali the Sahelo-Sudanian zone.

At the end of the phytochemical analysis of these crude aqueous and hydroethanolic extracts, the presence of alkaloids, flavonoids, tannins, saponosides, sterols, terpenoids, phenols,

anthraquinones, anthocyanins were revealed. These results confirm those of Bruneton in 1999 in France [28] and Cowan in Togo in 1999 [17], who demonstrated the presence of flavonoids, saponosides and tannins in extracts of *Cassia alata*, a plant from the same family as *Acacia amythethophylla* (Fabaceae). The presence of these chemical groups is in agreement with the work carried out by El-Mahmoud and Doughari, in Nigeria in 2008 [29], which revealed the presence of flavonoids, tannins, phenols, alkaloids, saponosides and anthraquinones. in *Cassia alata* extracts. The results of phytochemistry show that certain plant species in Chad contain secondary metabolites which could be validly exploited in the formulation of improved traditional medicines. This assertion was confirmed by Dianmadje et al., who revealed in their study in N'Djamena in Chad in 2022 the presence of tannins, alkaloids, sterols, terpenoids and saponosides in *Tamarindus indica*, the same family as *Acacia amythethophylla* [30]. Similar results were obtained by Singou et al., in Mali in 2021 who characterized the presence of tannins, flavonoids, and anthocyanins in the pulp of *Acacia nilotica* [31]. Likewise, they confirm those of Koudoro. et al, in Benin in 2009 who had proven the presence of tannins, flavonoids, saponosides, alkaloids from the bark of Acacia polyacantha [27]. Okpanachi et al, in 2012 in Nigeria revealed the presence of flavonoids, saponosides, tannins, alkaloids and anthraquinones on the trunk of Acacia polyacantha [32]. These results also corroborate those of Kerharo and Adam in 1974; Maïga, in Mali in 1992; which revealed the presence of tannins, saponosides, sterols from *Entada africana* root extracts [33, 34].

Speaking of the search for the antibacterial effect which had constituted the second phase of the analysis, we studied the sensitivity of the selected bacteria with regard to conventional antibiotics, and at the same time with regard to the extracts of the plant studied, and the results were compared. Concerning the antibacterial effect of aqueous and hydroethanolic extracts of *Acacia amythethophylla*, few studies relating to the plant have been carried out, we then discuss the results in terms of metabolite identified during the analyzes and having an antibacterial effect confirmed by previous studies. Four bacterial strains were subjected to the sensitivity test against the extracts of the plant studied. These were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The extracts were tested at different concentrations. We reported the sensitivity of all strains to aqueous and hydroethanolic extracts of plants collected in the two study areas, at the minimum inhibitory concentration of 1.25 mg/mL. Except *Staphylococcus aureus* inhibits at the minimum inhibitory concentration of 5 mg/mL against the aqueous extract of Dourbali, and *Pseudomonas aeruginosa* inhibits at the minimum inhibitory concentration of 2.5 mg/mL against the hydroethanolic extract from Dourbali. These differences between the minimum inhibitory concentrations of extracts from plants collected in Pala and Dourbali could be explained by the climatic variation between the two harvesting areas. The environment has a direct impact on the composition of secondary metabolites, this corroborates data from the literature confirming that climate variability and changehave impacts on the practice of traditional medicine [35]. The antibacterial effect of the extracts used in this study could be explained by the presence of alkaloids, flavonoids, tannins, terpenes and saponosides [36].

Alkaloids, flavonoids, tannins, saponosides, terpenoids act in the same way as antibiotics from modern Defour pharmacies in Zaire in 1995 [37].

Flavonoids are good inhibitors of sortases (enzymes found in the cytoplasmic membrane of Gram-positive bacteria that catalyze all surface proteins, for example adhesins and internalins) and trace amounts of Rutin inhibit sortases A and B. Indeed, all strains of *Staphylococcus aureus* bacteria treated with Rutin showed a reduction in binding to fibrinogen, one of the host ligands to which the bacteria attach during infection Cushnie and Lam, 2011[37]. Flavonoids inhibit the release of virulence factors of this bacteria. Epigallocatechin prevents the secretion of coagulase and α-toxin Cushnie and Lam, 2011[37].

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 [39, 40].

The results of the quantitative analysis showed that the recipe had a higher total phenol content (0.86 ± 0.002 mgAGE/g) [41, 42]. Previous studies have shown the presence of phenolic compounds in different plant organs. Phenolic compounds are the main characteristic molecules of the plant kingdom. Their role is to defend plants against pathogens. They ensure human and animal nutrition and health. They are able to eliminate free radicals and inhibit lipid peroxidation by reducing hydroxyl, superoxide and pyroxyl radicals. They are also capable of trapping metal ions, as they have chelating properties. Polyphenols have significant antioxidant activity, greater for example than that of vitamins (Delattre et al., 2005; Haile & Kang, 2019; Stagos, 2019) [43, 44, 45].

The results obtained reveal a fairly high rate of resistance of the different bacterial strains isolated to aminopenicillins. These high rates of resistance to amoxicillin justify that aminopenicillins are no longer currently recommended for probabilistic treatment of urinary infections. These results are similar to other studies [46-47]. Of 158 samples received, 117 were women and 41 were men. This result was similar to that of Gonsu et al. in Yaoundé (Cameroon) who revealed that the female predominance of urinary infection is linked to the anatomicalconfiguration including the shortness of the urethra and the proximity of the genital and anal orifices [48].

Conclusion

The present study made it possible to highlight the richness of the hydroethanolic and aqueous extracts of the bark of the trunk of *Acacia amythethophylla* in compounds such as flavonoids, tannins, saponosides and alkaloids responsible for the antibacterial activities observed on the different strains of bacteria. isolated. The hydroethanolic and aqueous extracts of *Acacia amythethophylla* trunk bark could be a potential source of natural biomolecules to fight against bacterial infections. This study also made it possible to determine a high prevalence of bacteria frequently involved in urinary infections and highlighted high rates of resistance to aminopenicillins. The results are the link between modern medicine and ethnomedicine and show that plants could be used as alternatives to modern medicine.

In view of these results, we recommend carrying out expanded studies with local plants to better reconcile modern medicine and ethnomedicine in Chad.

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Contribution of the authors

All authors contributed significantly to the writing and editing of this manuscript. It has been seen and approved
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