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Phytochemical screening and antibacterial properties from extract of *Alchornea cordifolia* (Schumach. &Thonn.) Müll. Arg.

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Abstract
This study involved a survey on the use of extract of *Alchornea cordifolia* a medicinal plant used locally in Cameroon as traditional medicine for the treatment of infectious diseases.The stems of *Alchornea cordifolia* has submitted for chemical screening by conventional techniques focusing on color reactions and chemical precipitation. The susceptibility testing was realized at *Klebsiella pneumoniae* and *Escherichia coli* microbial strains. The macrodilution in liquid medium for 24 hours allowed evaluating its antibacterial activity on the *Salmonella spp*, *Escherichia coli*, *Klebsiella spp, Proteus mirabilis*, *Edwardsiella tarda, Pseudomonas spp*, *Serratia spp* and *Citrobacter* two reference strains: *Pseudomonas aeruginosa* and *Staphylococcus spp*. The phytochemical analysis showed the presence of flavonoids, anthocyanins, tannins, alkaloids, polyphenols and cardiac glycosides. The best inhibitory activities were obtained with *Escherichia coli* at 3.125 mg/mL of MIC, with a ratio MIC/MBC of 2, and at 6.25 mg/mL of MBC; with a ratio MBC/MIC of 2. The minimal inhibitory, bactericidal concentration varies between 3.125 and 100 mg/mL. The present study revealed the antimicrobial potentials of hydroalcoholic extract of *Alchornea cordifolia* on eleven microbial strains. A thorough study would be desirable for development of new drugs in the future.

Keywords: *Alchornea cordifolia,* phytochemical screening, antibacterial activities

1. Introduction
Infections due to pathogenic bacteria and fungi represent a critical problem to human health [1]. In Cameroon, infectious diseases are among the most commonly reported and the largest cause of death [2]. Pathogenic strains of bacteria and fungi are especially prevalent in immune compromised patients, causing many deceases annually. Hence, alternative therapies are urgently needed to treat patients affected by pathogenic microorganisms [3]. In additional, widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones and the lack of effective new therapeutics exacerbate the problems [4]. The treatment of patients with bacteremia nowadays is becoming more complicated, because the increasing microbial resistance against the limited number of available commercial antimicrobial agents [5].
The search for new antimicrobial compounds is particularly important and Strategies to improve the current situation include research to find new antibacterial agents of plant origin. Plants have a great potential for the production of new drugs useful for humanity [6, 7].
In African countries, about 80% of the population depends on traditional medicine for their treatment, because it is readily available and affordable economic-wise [8] and The World Health Organization (WHO) estimates that nearly 70% of the world population depend on traditional medicine, especially medicinal plants, for their primary health care needs [9]. In an effort to discover new lead compounds many research groups have screened plant extracts to detect secondary metabolites with relevant biological activities [10]. The antimicrobial and biological activities of many plants have been attributed to various phytochemicals such as alkaloids, glycosides, tannins and saponins [11].
The genus *Alchornea* belongs to the spurge family Euphorbiaceae, which contains about 7500 species in all parts of the world, including trees, shrubs and herbs [12]. The plant leaf extracts have been reported to possess antimicrobial activity and its spectrum of activity has been shown to include the Gram negative bacteria and the Gram positive bacteria [13, 14, 15]. It antifungal activity was reported [16]. This present study has been conducted in perspectives to investigate the phytochemical composition and antibacterial properties from extract of *Alchornea cordifolia*.

2. Material and methods

2.1. *Plant materials and preparation of extract*

Stems of *Alchornea cordifolia* consisted plant material. The harvest had been made in the region Centre, Akounou village, 40 Km from Yaounde. The stems harvested in November 2014, and dried in the sun for 2 weeks had been pulverized by a mechanical grinder.

Fig 1: Picture of *Alchornea cordifolia*

2.2. *Phytochemical screening*

5800 g of Powder stems of *Alchornea cordifolia*, soaked at room temperature in 17,400 mL of ethanol (70:30 v/v in water). The filtrate was collected and the residue was added 5800 mL of solvent to further extraction. The two filtrates were combined in one volume. Filtration and extract concentration were performed with a whatman filter paper N°1 and a Rotavapor (HEIDOLPH, HEIZBAB® at 45°C and 200 bars) respectively. The crude extracts thus obtained were subject to the identification by precipitation reactions and staining according to the methodology of the phytochemical screening performed by respective conventional reactions [18]

Table 1: Usual methods of phytochemical screening

Secondary metabolite	Reagent of identification	Indicator
Alkaloid	$HgCl_2$ and KI (Mayer)	Creamy white precipitate or White-yellow color
	Picric acid (Hager) I_2 and KI (Wagner)	Reddish-brown precipitate Creamy white precipitate
Polyphenols	$FeCl_3$	Greenish color
Flavonoids	NaOH and H_2SO_4	Orange color
Anthocyanin	$H_2SO_4$and NH_4OH	Purplish blue
Catechuk tannin	Formalin and HCl	Gelatinous precipitate
Gallic tannin	Lead acetate and $FeCl_3$	Blue-black color
Mucilage	Absolute ethanol	Flocculent precipitate
Saponosides	Foam index	Persistent foam
Steroids and H_2SO_4	Acetic anhydride acid	Color from purple to blue or green
Resins and H_2SO_4	Acetic anhydride acid	Yellow color
Cardiac glycosides $FeCl_3$+ H_2SO_4	Glacial acetic acid and	Greenish or brown color
Quinones	H_2SO_4	Red color
Coumarins	$FeCl_3$ and HNO_3	Green or blue that turns yellow

2.3. Antibacterial evaluation

2.3.1. Preparation of extract solutions

Plant extracts solutions were prepared at a concentration of 50000 µg/mL. The dissolving solvent is Mueller Hinton broth.

2.3.2. Sensibility test

The sensitivity test was performed on two microorganisms: *Klebsiella pneumoniae* T 81 and *Escherichia coli* 00172. The plant extract was used at concentration of 500 mg/mL. This test was mainly based on susceptibility testing. From a pure culture of 24 hours on plain agar, a suspension is prepared in 5 mL of saline by taking 3-4 colonies with a sterile loop to obtain a bacterial inoculum equivalent opacity to the standard 0.5 scale Mc Farland (use a white-black striped paper and a scale indicator). This is followed by seeding by flooding the agar surface using the suspension prepared above, and then reabsorbs excess suspension using a sterile pipette "Pasteur". At this stage, the preparation is covered and allowed to incubate on the bench for 10 minutes. When ten minutes elapsed, the antibiotic discs are deposited on the surface of the inoculated agar (6 discs/box approximately 30 mm between the discs and 15 mm from the edge of the box) and the whole is led to the incubator at 37° C under aerobic conditions. The incubation time is 18 to 24 hours for both ranges after which the diameter of inhibition is measured in millimeter diameter of the disc included [19].

2.3.3. *Preparation of Bacterial Inoculum*

For each bacterial strain, a 0.5 Mc Farland suspension is made in physiological saline. This suspension corresponds to a concentration of approximately 1 million bacteria / mL.

2.3.4. *Determination of minimum inhibitory concentrations (MIC)*

Macrodilution technique in liquid medium was used [20]; Mueller Hinton broth (1000 µL) was introduced into 14 tubes making a range of dilution. The first volume of the extract was from a stock solution S, previously filtered on sterile membrane. Then 1000 µL of the stock solution S, being concentrated at 400 mg / mL, were introduced into the first tube of the dilution range. As result, serial dilutions were in Mueller Hinton broth, so as to obtain a concentration range between 400 mg / mL and 195.10^{-3} mg / mL of plant extract. Then 15 µL of bacterial inoculum was added to each tube of the dilution range (except the controls), and then incubated at 37 °C. After 18 to 24 hours, the turbidity was visually evaluated, and the tubes were centrifuged at a speed of 5000 revolutions / minute for 5 minutes. The MIC of each test sample was derived from the first tube of the range within which any visible growth has not occurred.

2.3.5. *Determination of minimum bactericidal concentrations (MBC)*

The minimum bactericidal concentration (MBC for bacteria) is the minimum concentration corresponding to the lowest concentration of a substance capable of killing more than 99.9% of bacterial inoculum or initial (less than 0.1% of survivors) after 18 at 24. hours of incubation at a temperature of 37 °C [21]; and MBC determination was based on the subculture of bacterial inoculum from the MIC on nutrient agar [22].

In each of the tubes in which visible growth was not observed and the control tube used in determining the MIC [22], samples were taken and then streaked on Mueller Hinton agar plates which were then incubated for 18-24 hours at 37 °C. CMB of each extract was derived from the first dilution (concentration) at which no culture was observed on Mueller Hinton agar.

3. Results

3.1. Phytochemical screening

Phytochemical analysis of secondary metabolites tested in the stem ethanol extracts of *Alchornea cordifolia*.

Table 2: Results of phytochemical analysis

Secondary metabolite	Conclusion
Flavonoids	+
Anthocyanins	+
Tannins	+
Alkaloids	+
Steroids	-
Resins	-
Polyphenols	+
Saponosides	-
Cardiac glycosides	+
Coumarins	-
Quinones	-
Mucilage	-

Legend: *+ Presence -Absence*

3.2. Antibacterial properties from extract of *Alchornea cordifolia*

Table 3. Minimum concentrations of Alchornea cordifolia

	MIC mg/mL.	MBC mg/mL	R=MIC/MBC
Escherichia coli	3,125	6,25	2
Serratia spp	50	100	2
Citrobacter spp	25	200	8
Klebsiella spp	12,5	25	8
Pseudomonas spp	25	100	4
Pseudomonas aeruginosa	25	50	2
Edwasiella tarda	12,5	25	2
Salmonella spp	12,5	100	8
Staphylococcus aureus	25	-	-
Proteus mirabilis	12,5	100	8

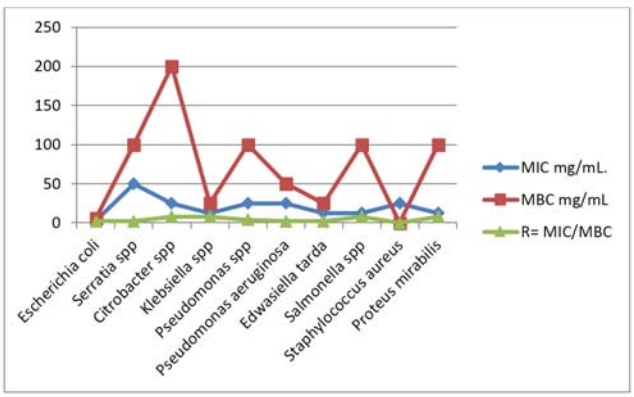

Fig 2: Evolution of minimum inhibitory concentrations (MIC), bactericidal (MBC) and their ratio (R).

Legend: MIC: Minimum Inhibitory Concentration;
MBC: Minimum Bactericidal Concentration
R: Ratio MBC/MIC.

At the end of these results, we note that MIC was obtained on all microbial strains studied, and they vary from 3.125 mg / mL (*Escherichia coli*) to 50 mg / mL (*Serratia spp*). For MBC vary from 6.25 mg / mL (*Escherichia coli*) to 200 mg / mL (*Citrobacter spp*). However the microbial strains namely *Staphylococcus aureus* showed no MBC.

4. Discussion

The result of the phytochemical screening (Table 2) of the ethanolic extract of *Alchornea cordifolia* stems showed the presence of flavonoids, anthocyanins, tannins, alkaloids,

polyphenols and cardiac glycosides. In general, the most active plant extracts from the Euphorbiaceae family displayed the presence of tannins and flavonoids; these facts are in consonance with the polyphenols content of some plants from the Euphorbiaceae family that showed condensate and hydrolysable tannins, flavonoids, among others, responsible for their biological activities [23]. The presence of flavonoid in the plant suggests that it can be used as anti-spasmodic, anti-fungal and anti-bacterial drug plant. These findings confirm the reason for the use of the plant in the treatment of diarrhea, spasmodic bronchitis and other microbial infections [24].

Tannins have an antibacterial effect by precipitating their proteins making them unavailable their nutritional proteins [25]. This general weak antibacterial propriety on the tested organisms may be due to the absence of other compounds such as the alkaloids, terpenes that could have acted in synergy with the tannins that were richly present in the studied plant. Previous studied have shown antibacterial properties some of these secondary metabolites, including flavonoids, saponins, steroids and terpenes [5]. Ulanowska *et al.* [26] evoke interest remarkable flavonoids against microbial diseases. In the same manner, the antimicrobial action of alkaloids could be throughout intercalation with cell wall and/or DNA constituents; while, terpenoids display their action through membrane disruption mechanisms [27]. Alkaloids have been shown to possess antibacterial [38].

Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared [29].

The presence of secondary metabolites in *Alchornea cordifolia* has been reported to be responsible for their antibacterial properties [20].

Ours study demonstrated different concentrations of *Alchornea cordifolia* at different microbiological strains. The best inhibitory activities were obtained with *Escherichia coli* at 3.125 mg/mL of MIC, with a ratio MIC/MBC of 2, and at 6.25 mg/mL of MBC and MFB; with a ratio MBC/MIC of 2. The same ratio (MIC/MBC) has obtained with *Serratia spp, Klebsiella spp, Pseudomonas aeruginosa* and *Edwasiella tarda*. These results are close of that obtained by Okwu and Ukanwa[30] to whom that the isolated compound successfully inhibited *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia* and *Staphylococcus aureus.*

Some fractions, notably those containing phenolics and terpenoids, exhibited significant activity against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* [31].

Antimicrobial substances are considered as bactericidal agent when the ratio MIC/MBC ≤ 4 and bacteriostatic when the ratio MBC/MIC is > 4 [32]. For most germs tested in the present study the ratio is ≤ 4.

Conclusion

This study showed that hydroalcoholic extract of *Alchornea cordifolia* stems possess an antibacterial activity against the test bacteria species. This specie constitutes a potential antibacterial activity that can be explored as remedy for human bacterial infections according ours results and the preceding studies of different solvents and plant parts. These results also provide valuable information for further detailed studies of active compounds, necessary for the development of new drugs in the future.

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