



## In vitro Assessment of Antibacterial and Antioxidant Activities of a Congolese Medicinal Plant Species *Anthocleista schweinfurthii* Gilg (Gentianaceae)

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### ABSTRACT

The plant species *Anthocleista schweinfurthii* is eaten by bonobos, an endemic pygmy chimpanzee of Democratic Republic of the Congo and also used in folk medicine to treat bacterial diseases. To provide a scientific basis to traditional uses, the plant species was screened for its antibacterial and antioxidant potential.

Antibacterial activity was assessed by minimum inhibitory concentration method. The presence of phytoconstituents was evaluated qualitatively. *A. schweinfurthii* extracts displayed interesting antibacterial and antioxidant activities. The Gram positive bacteria *S. aureus* ATCC 33591 were more sensitive to *A. schweinfurthii* than the Gram negative *E. coli* ATCC 27195. The stem bark extracts and n-hexane fraction of the leaves were found to be biologically active against *Staphylococcus aureus* ATCC 33591 strains (MIC  $\leq$  62, 5  $\mu$ g/mL) while, the bioactivity of dichloromethane, ethyl acetate and methanol soluble fractions of the leaves was moderate (MIC  $>$  100  $\mu$ g/mL). The methanol extract of the stem bark displayed interesting bioactivity (MIC = 62,5  $\mu$ g/mL) while the leaves based extracts and the n-hexane, the dichloromethane and the ethyl acetate fractions of the stem bark of *A. schweinfurthii* displayed moderate activity against *E. Coli* ATCC 27195 strains (MIC  $>$  100  $\mu$ g/mL). Methanol extract of *A. schweinfurthii* displayed also interesting free radical Scavenging activity (IC<sub>50</sub>  $<$  10  $\mu$ g/mL). Phytochemical analysis revealed the presence of total polyphenols, alkaloids, terpenes and steroids while, anthocyanins, leuco-anthocyanins, flavonoids, tannins, coumarins, quinones and saponins were absent in the plant extracts.

Antibacterial and free radical scavenging efficacy shown by this plant provides a scientific basis and thus, validates its traditional use as phytomedicine. Isolation and purification of different phytochemicals may further yield significant antibacterial and antioxidant agents.

**Keywords:** Traditional medicine, *Anthocleista schweinfurthii*, bacterial infections, antioxidant activity, Democratic Republic of the Congo

### INTRODUCTION

Microbial diseases constitute a major public health problem worldwide, and are currently the world's leading cause of death because of the emergence of multidrug resistance among several pathogenic agents to drugs commonly used to treat such diseases [1].

The increase of antibiotic resistance by the pathogenic microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective drugs for the control of infectious diseases. Several reports have shown that the medicinal plants constitute a great source of biologically active drugs for the

control of pathogenic organisms because of the enormous chemical and structural diversity of plant derived secondary metabolites [1-6].

Microbial infections, especially due to *Staphylococcus*, *Streptococcus* and *Pseudomonas* species, and the presence of oxygen free radicals, are known impediments to wound healing [7]. It is also known that, infections are recurrent pathologies of the Sick Cell Disease (SCD) and remain the leading cause of death in children patients. In SCD, *Staphylococcus aureus* is the bacteria most implicated in septicemia and osteomyelitis, while some

serotypes of *Escherichia coli* (K1) are able of causing very serious neonatal infections that are potentially complicated by meningitis or septicemia [8, 9]. Any agent capable of eliminating or reducing the number of microorganisms, as well as reducing the level of reactive oxygen species (ROS), may facilitate the wound healing process or useful for SCD patients. It then becomes necessary to search for new antimicrobial and antioxidant drugs, especially those that would be cheap and thus easily affordable by poor population.

The present study was performed with the aim of evaluating the antimicrobial and antioxidant activities of different extracts of *Anthocleista schweinfurthii* Gilg (Synonym: *Anthocleista laurentii* De Wild.; *A. magnifica* Gilg; *A. niarniamensis* Gilg; *A. oubanguiensis* Aubrév. & Pellgr.; *A. pynaertii* De Wild.; *A. squamata* De Wild. & T.Durand; *A. stuhmannii* Gilg; *A. gigantean* Gilg; *A. insulana* S.Moore; *A. kamerunensis* Gilg). *Anthocleista schweinfurthii* Gilg is a big tree originated from tropical regions. Leaves, stem and root barks of this plant are eaten by bonobos, an endemic pygmy chimpanzee of Democratic Republic of the Congo and also used in folk medicine to treat several disorders (malaria, cancers, venereal diseases, bacterial diseases) [10].

## MATERIALS AND METHODS

### *Plant material collection and identification*

The tested plant materials (stem bark and leaves) used in this study were collected in Democratic Republic of the Congo during a field work in March 2011 and were authenticated by Mr Jonas Zamena of the INERA (Institut National d'Etudes et Recherches Agronomiques). Vouchers specimens are on deposit at the INERA Herbarium of the Faculty of Science (Université de Kinshasa).

### *Extraction and chemical screening*

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol (EtOH) and water (100 mL x 2) for 48 hours. Chemical screening was done in aqueous and organic extract according to a well known protocol as previously reported. Fractions were filtered and concentrated to dryness under reduced pressure using a rotary evaporator [11].

### *Preparation of methanol extract and increasing polarity extracts*

Each plant powder (100 g) was macerated in methanol 80% (1L x 2) for 48 hours. After filtering the mixture, the aqueous-methanolic filtrate was concentrated under reduced pressure using a rotary evaporator. The methanolic extract was suspended in distilled water and sequentially partitioned with n-hexane, dichloromethane, ethyl acetate, ethanol, and methanol (1:1, v/v) three times at room temperature. The resulting fractions were evaporated to dryness on an evaporator apparatus. All extracts were stored at +4 °C.

### *Antioxidant assay*

The DPPH free radical (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay was performed as previously reported [12-14]. The radical scavenging activity of extracts for DPPH free radical was measured based on the principle of the reduction of the DPPH radical to a yellow-colored compound (diphenyl picrylhydrazin) in the presence of an antioxidant; the extent of the reaction depending on the hydrogen donating ability of the antioxidant. Briefly, a 100 µM solution of DPPH radical in methanol was prepared. 3,5 mL of this solution was added to 0,5 mL solution of each extract in methanol at concentrations ranging from 0,1 to 1 mg/mL, thus obtaining the desired final concentrations in the reaction mixture. The mixture was shaken vigorously and

incubated in the dark at room temperature for 30 min. The absorbance was measured at 515 nm using a spectrophotometer SP- 1105 Brand model. Methanol was used as a blank. The control solution consists of 0,5 mL of methanol and 3,5 mL of DPPH radical solution. The antiradical activity of a sample (calculated by the following formula) is given as percentage of reduced DPPH free radical: %I = [(OD control - OD sample)/OD control] × 100. The IC<sub>50</sub> value (µg/mL) is the effective concentration at which DPPH radicals were scavenged by 50%. L-ascorbic acid was used as positive control. Duplicate analyses were run for each extract.

### *Antibacterial evaluation*

#### *Microbial strains*

The activity of the plant samples was tested toward *Staphylococcus aureus* (*S. aureus* ATCC 25923) and *Escherichia coli* (*E. coli* ATCC 25922). The tested strains were obtained from the American Type Culture Collection (ATCC, Rockville MD, USA).

#### *Determination of Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

MIC was determined by broth micro-dilution method as previously reported [1]. The inocula of microorganisms were prepared from 24 hours old broth cultures. The absorbance was read at 600 nm and adjusted with sterile physiological solution to match that of a 0.5 McFarland standard solution. From the prepared microbial solutions, other dilutions with sterile physiological solution were prepared to give a final concentration of 10<sup>6</sup> colony-forming units (CFU) per milliliter. Stock solutions of the extracts were prepared in 0.1% (v/v) aqueous tween 80 (Fisher chemicals) at concentrations of 1 mg/mL. The two-fold serial dilutions in concentrations of the extracts were prepared in Mueller Hinton Broth (MHB) (Conda, Madrid, Spain) to give final concentrations ranging from 250 to 1.95 µg/mL.

An aliquot (10 µL) of a 10<sup>6</sup> CFU/mL overnight culture was added to wells of a sterile 96-well micro-plate titer. The positive control wells contained MHB+ bacteria suspension without plant extract while negative control wells contained MHB only. The minimum inhibitory concentration (MIC) was determined as the lowest plant extract concentration at which no growth were observed after 24 hours. MTT (30 µL) in aqueous solution (0.01%) was used to evaluate the micro-organism viability. For minimum bactericidal (MBC) determination, 10 µL was taken from each well of complete inhibition of bacterial growth after incubation and spot inoculated on MHB and incubated for 72 hours at 37 °C. The concentration at which no growth observed on subculture was determined as the MBC.

## RESULTS AND DISCUSSION

### *Chemical screening*

The results of chemical screening *Anthocleista schweinfurthii* are presented in Table 1. It is deduced from the table 1 that leaves and stem bark of *Anthocleista schweinfurthii* contain total polyphenols, alkaloids, terpenes and steroids. However, we also note that compounds such as anthocyanins, leuco-anthocyanins, flavonoids, tannins, coumarins, quinones and saponins are not found in the two plant organs.

The presence of various secondary metabolites in the plant could justify its medical use. Indeed, *A. schweinfurthii* is reported to treat bacterial infections [10]. Compounds, which are significantly present in the plant, are well known for their large spectrum of pharmacological properties, including antimicrobial (alkaloids) and antioxidant (polyphenols) activities [15-16]

Table 1: Chemical screening of *Anthocleista schweinfurthii*

Chemical groups	<i>Anthocleista schweinfurthii</i>	
	Stem bark	Leaves
Total polyphenols	+	+
Anthocyanins	-	-
Leuco-anthocyanins	-	-
Flavonoids	-	-
Gallic tannins	-	-
Catechic tannins	-	-
Coumarines	-	-
Quinones	-	-
Alkaloids	+	+
Saponins	-	-
Terpenes and steroids	+	+

### Extraction yields

Extraction yields of *Anthocleista schweinfurthii* stem bark (left) and leaves (right) are given in Figure 1.

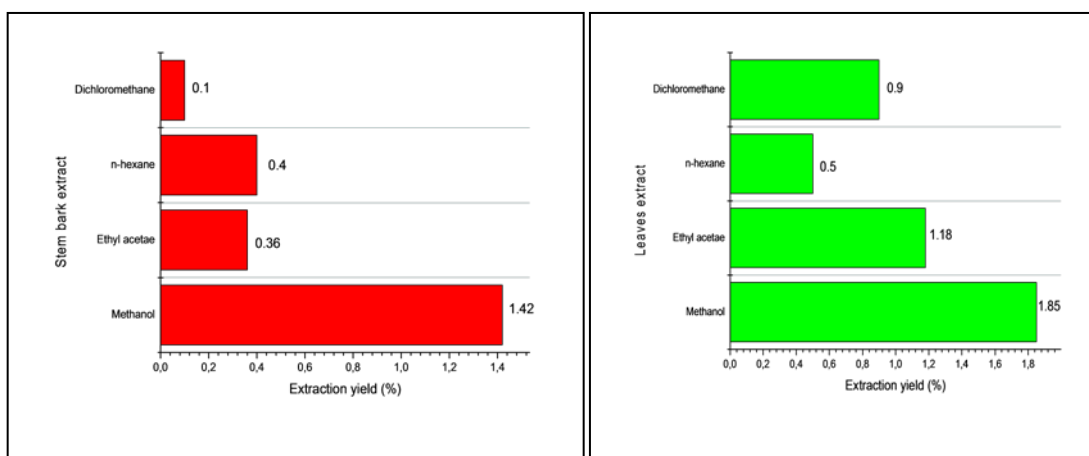


Figure 1: Extraction yields of *Anthocleista schweinfurthii* stem bark (left) and leaves (right)

According to the results presented in figure 1, it is clearly shown that the unpolar solvents have fewer yields than polar ones. Indeed, methanol gives a high output; and poor yield is obtained with dichloromethane. This reveals that the abundant metabolites in *Anthocleista schweinfurthii* are those which pass easily through the polar solvents. The extraction yields of plant are 1.42% (methanol), 0.4% (n-hexane), 0.36% (ethyl acetate) and 0.1% (dichloromethane) for the stem bark and 1.85% (methanol), 1.18% (ethyl acetate), 0.5% (n-hexane) and 0.9% (dichloromethane) for the leaves. Leaves were found to contain more secondary metabolites than the stem bark. This difference in the compounds content of plant organs can be explained by the fact that leaves are the site of biochemical pathways of photosynthesis and naturally occurring secondary metabolites synthesis [17].

### Antibacterial activity

The antimicrobial activity of extracts from *Anthocleista schweinfurthii* against *Staphylococcus aureus* and *Escherichia coli* strains was determined. The results are shown in Tables 2 and 3.

From the table 2, it can be deduced that stem bark extracts and n-hexane fraction of the leaves are biologically active

against *Staphylococcus aureus* ATCC 33591 strains (MIC  $\leq$  62,5  $\mu$ g/mL) while, the bioactivity of dichloromethane, ethyl acetate and methanol soluble fractions of the leaves was moderate (MIC  $>$  100  $\mu$ g/mL). These results indicate that the antibacterial activity the stem bark is greater than that of the leaves. This activity was found to be bactericidal.

From the table 3, it can be deduced that methanol extract of the stem bark displayed interesting bioactivity (MIC = 62,5  $\mu$ g/mL), while the leaves based extracts and the n-hexane, the dichloromethane and the ethyl acetate fractions of the stem bark of *Anthocleista schweinfurthii* displayed moderate activity against *E. Coli* ATCC 27195 strains (MIC  $>$  100  $\mu$ g/mL).

The present study revealed that the Gram positive bacteria *S. aureus* were more sensitive to *Anthocleista schweinfurthii* than the Gram negative *E. coli*. The higher sensitivity of Gram-positive bacteria could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier. Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components make the cell wall impermeable to lipophilic solutes while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of 600 Da [18].

The present findings indicate that *Anthocleista schweinfurthii* could serve as promising source of antimicrobials. Historically, Plants and microbial pathogens have been living together for many centuries. In such co-evolution, plants develop elegant and numerous biochemical defense strategies to counter the microorganisms attack by producing specialized secondary metabolites that have toxic effect on the microbes. These bactericidal/bacteriostatic and anti-infective naturally occurring compounds are use as medicines [19]. Indeed, despite the fact that

plant pathogenic microorganisms have played a key role in the early evolution of the secondary metabolites diversity, there is little chance for a microbe to gain resistance from a plant as it is known for antibiotic-producing microbes which possess genes protecting them from the toxic effects of these compounds. Like microbial antibiotics, plant antimicrobial secondary metabolites could kill pathogenic agent via a non-species specific mechanism such as disrupting microbial cell membranes or inhibiting quorum sensing phenomena [20, 21].

Table 2: Inhibitory effect of *Anthocleista schweinfurthii* extracts against *Staphylococcus aureus* ATCC 33591 (expressed as the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC).

Used parts	MIC/MBC ( $\mu\text{g/mL}$ )			
	<i>Anthocleista schweinfurthii</i> extract			
	n-hexane	Dichloromethane	Ethyl acetate	Methanol
Stem bark	15,625/31,5	31,25/62,5	1,953/5,859	62,5/125
Leaves	62,5/125	125/250	125/250	125/250

Table 3: Inhibitory effect of *Anthocleista schweinfurthii* extracts against *E. Coli* ATCC 27195 (expressed as the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC).

Used parts	MIC/MBC ( $\mu\text{g/mL}$ )			
	<i>Anthocleista schweinfurthii</i> extract			
	n-hexane	Dichloromethane	Ethyl acetate	Methanol
Stem bark	125/250	125/125	125/250	62,5/125
Leaves	125/250	125/250	125/250	125/250

### Antioxidant activity

The radical scavenging activity of different fractions is given in Table 4.

Table 4: Radical scavenging activity of some fractions from *Anthocleista schweinfurthii*

Fraction	IC <sub>50</sub> ( $\mu\text{g/mL}$ )	Free radical Scavenging Activity
L-Ascorbic Acid (Positive control)	0.56 $\pm$ 0.21	1.79
Leaves MeOH extract	1.20 $\pm$ 0.05	0.83
Stem bark MeOH extract	3.90 $\pm$ 0.32	0.26

MeOH methanol

As it can be noticed from Table 4, methanol extract of *Anthocleista schweinfurthii* displayed interesting free radical Scavenging activity (IC<sub>50</sub> < 10  $\mu\text{g/mL}$ ). But, compared to the positive control (high antioxidant activity) this activity was weak while leaves extract displayed high activity than the stem bark.

The production of free radicals within the cells is the common biochemical pathway of the chronic and infectious

diseases [22]. An herbal drug presenting at the same time antibacterial and antioxidant properties can be useful for the management of such diseases.

Moreover, humans and great apes (bonobos, chimpanzees, gorillas, and orangutans) share a common gut anatomy. Although, some diseases that cause countless deaths in humans are ineffective or have minor non disturbing effects in apes. Indeed,

humans and great apes, when displaying symptoms of illness could alter their foraging to ingest non-nutritive chemical as diet (pharmacophagy). *Anthocleista schweinfurthii* as a plant species consumed by bonobo, by displaying antioxidant activity could justify the role of animal self-medicative behaviour as source of possible epigenome modulators and may aid in the control of infectious diseases and SCD [23].

## CONCLUSION

The present study provided evidence for the antimicrobial and antioxidant activities of studied plant extracts, and brings supportive data for future investigations that will lead to the use of standardized herbal medicine from *Anthocleista schweinfurthii* bioactive extracts in oxidative stress induced disease such as SCD and antimicrobial therapy.

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