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## Preliminary phytochemical screening and antimicrobial evaluation of leaves and barks extracts from *Cola anomala* (Schott and Endlicher)

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### Abstract

*Cola* is a tropical African genus that belongs to Sterculiaceae. The aim of this study is to investigate the preliminary phytochemical analysis and antimicrobial evaluation of the leaves and barks from *C. anomala*. The phytochemical identification of secondary metabolites in different extracts of the plant was focused on the classic methods of screening. The extraction of extract was carried out with maceration for 72 hours in ethanol-water (70:30 v/v) and the tannins by Gédér's method. Minimum Inhibitory Concentrations were determined by micro dilution method by preparing the different dilutions of extract and tannins from leaves and barks of *C. anomala* on *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella flexneri*; as bacterial strains. Ampicillin was taken as reference drug. The preliminary phytochemical analysis showed the presence of catechin tannins, alkaloids, polyphenol compounds, saponosides and flavonoids. All extracts and tannins showed an inhibition against the strains. The Crude extract from barks was the most active with a minimal inhibitory concentration varied between 0.625 mg/ml to 1.25 mg/ml respectively against *Staphylococcus aureus* and the other strains (*Salmonella enterica*, *Shigella flexneri*, and *Enterococcus faecalis*). MBC values of extracts varied from 1.25 to 5 mg/ml. Overall, for all treated strains, tannic extracts showed bactericidal potential than crude extracts.

**Keywords:** *Cola anomala*, secondary metabolites, MIC, MBC, tannins

### 1. Introduction

The search for newer antimicrobial agents from various sources has become imperative because of the emergence of resistance strains of microorganisms against orthodox antibiotics especially difficulty to treat infections from resistant strains of bacteria [1]. Plants possess economically and therapeutically valuable metabolites; therefore, plant products gained extensive importance to be used for medicinal purposes [2]. In recent decades, due to the large amount of research on Phytochemistry and Pharmacognosy, natural products from plant sources have gained particular importance in the treatment of infectious diseases [3].

The humid tropics of West and Central Africa contain a wealth of forest resources [4]. Three species of *Cola* are known in Cameroon as non-timber forest products, they are cultivated by small farmers in association with *Theobroma cacao* or coffee in the centre and south regions (*C. acuminata*), west and north regions (*C. anomala*) and south-west and littoral regions (*C. nitida*) [5]. *Cola* nuts have for hundreds of years been widely traded in Africa, especially in the trans-Saharan trade routes.

*Cola anomala* is a tropical tree species of West and Central Africa that belongs to the Malvaceae (formerly Sterculiaceae) family. It is cultivated by subsistence farmers as shade over cacao and/or coffee plants, and for its edible nuts. The plant is used in South part of Cameroon against asthma and caught [6].

However few investigations have mentioned the phytochemical composition and antimicrobial evaluation of *C. anomala*. Thus, the aim of this study is to investigate the preliminary phytochemical analysis and antimicrobial evaluation of the bark and leaf extracts from *C. anomala*.

### 2. Materials and Methods

#### 2.1 Plant material

The leaves and barks of *C. anomala* were harvested in Baboate (West Cameroon) in January 2017. They were then identified at Cameroon National Herbarium (CNH) to confirm its Identity. The plant material was confirmed by comparison to voucher specimen n°48707/HNC. Sample was dried in the shade for two weeks then pulverized by a mechanical grinder.

## 2.2 Extraction of condensed tannins

Extraction of the condensed tannins was carried out according to the method described by Gédír *et al* (the aqueous extract of leaves of *C. anomala* was treated with Sodium chloride Brine. The residual solution was then treated with ethyl acetate. The collected organic phase was treated with anhydrous sodium sulphate) [7]. Samples from leaves, 300 g of powder were macerated 3 times successively for 72 hours in 2 liters of a mixture of ethanol-water (70:30 v/v). Obtained macerates were filtered with Whatman paper No. 4. The first part was dried in an oven at 40 °C to obtain the crude extract. The second one was concentrated in a Rotavapor to remove the ethanol and obtain the aqueous extract.

## 2.3 Phytochemical screening

### 2.3.1 Test of alkaloids

Alkaloids were tested by Dragendorff and Mayer's reagents. To 1ml of each extract, 2 ml of Dragendorff's reagent was added and mixed. To this 2 ml of dilute HCl was added [8]. Formation of an orange colored precipitate indicates the presence of alkaloids. As for Mayer's reagent, the apparition of a yellowish-white precipitate has shown the presence of alkaloids [9].

### 2.3.2 Test of flavonoids

Iso-amyl alcohol was added to the plant extract with magnesium shaving and a few drops of hydrochloric acid. The appearance of a pink or red color indicated the presence of flavonoids [10].

### 2.3.3 Test of phenolic compounds

FeCl<sub>3</sub> was added to the extract. The appearance of a blue-black color indicated the presence of phenols. The test was confirmed with the appearance of a white precipitate when adding lead acetate [11].

### 2.3.4 Test of saponosides

To search for saponosides, we poured 10 ml of the extract into a test tube. The tube was stirred for 15 seconds and then allowed to stand for 15 minutes. Persistent foam height greater than 1 cm indicated the presence of saponosides [12].

### 2.3.5 Test of tannins

Five grams of sample powder are added to 100 ml of boiling water. After 15 min, the suspension is filtered and rinsed. Hydrolysable gallic tannins are evidenced by adding 15 ml of Stiasny reagent to 30 ml of the 5% infusion. After heating in a water bath at 90 °C during 15 min, the mixture is filtered and saturated with 5 mg of sodium acetate, and then 1 mL of a solution of 1% FeCl<sub>3</sub> is added. The appearance of a blue-black tint indicates the presence of gallic tannins. The non-hydrolyzable catechin tannins are characterized by the addition of 1 ml of conc. HCl to 5 ml of the previously prepared infusion. The mixture is boiled for 15 min. In the presence of catechin tannins, a red precipitate, insoluble in isoamyl alcohol, is formed [13].

## 2.4 Antimicrobial study

### 2.4.1 Microbial strains

Six microbial (bacteria) strains were obtained from (Centre Pasteur). Overall, two were positive grams (*Enterococcus faecalis* ATCC 51298 and *Staphylococcus aureus* ATCC 43300) and two negative grams (*Salmonella enterica* NR4311, *Shigella flexneri* NR518).

### 2.4.2 Preparation of Bacterial Inocula

The turbidity of the suspension obtained was adjusted to that corresponding to the Mc Farland standard range (3 x 10<sup>8</sup> CFU/ml). Subsequently these suspensions were diluted with sterile distilled water to obtain a concentration of 5 x 10<sup>6</sup> CFU/ml. Petri dishes containing MHA were seeded by flooding with inoculum suspensions titrated at 5 x 10<sup>6</sup> CFU/ml. Then each sterile paper disks of 6 mm diameter (Whatman No. 3) were impregnated with 10 µl of extracts (5 mg/ml). Then these discs were deposited on Petri dishes. The dishes were incubated at 37 °C for 24 hours.

### 2.4.3 Determination of minimum inhibitory concentrations (MIC)

We used the method of microdilution in liquid medium following the protocol M07-A9 [14].

Extract solutions (concentration of 20 mg / ml) were prepared with a 95: 5 (v/v) MHB-ethanol mixture. Ampicillin was prepared at 400 µg / ml in MHB. The turbidity of the suspension obtained was adjusted to that of the 0.5 Mc Farland standard (1.5 x 10<sup>8</sup> CFU/ml). Subsequently, these suspensions were diluted with Muller Hinton Broth (MHB) in order to obtain a suspension titrated at 10<sup>7</sup> CFU/ml. 100 µl of MHB were introduced into all the microcupules of the plate. At the first line, 100 µl of extract were added and homogenized to have a volume of 200 µl. From these microcupules, a series of 6 geometric dilutions of order of 2 was carried out. Then 100 µl of an inoculum suspension titrated at 10<sup>7</sup> CFU/ml were introduced into all the wells, to obtain a final inoculum at 5 x 10<sup>6</sup> CFU/ml. Plates were incubated at 37 °C for 24 hours. The tests were done in duplicate. The same procedure was carried out with ampicillin (diluted 6 times) from the first line (100 µg / ml) to the sixth line (1.56 µg / ml).

For control, 100 µl of bacterial suspension were added in 100 µl of liquid medium.

### 2.4.4 Determination of minimum bactericidal concentrations (MBC)

The MBC is the lowest concentration of antibiotic leaving after 18 h of incubation a percentage of survivors < 0.01% of the starting inoculum. To determine the CMB, we seeded all the cups preceding the cup that corresponds to the MIC. The microplates were incubated at 37 °C for 24 hours. The lowest concentration at which no visible growth was observed was considered the minimum bactericidal concentration.

## 3. Results

### 3.1 Preliminary phytochemical screening

**Table 1:** Results of preliminary phytochemical screening

Secondary metabolites	CEL	CEB	TEL	TEB
Alkaloids	+	+	-	-
Catechin tannins	+	+	+	+
Flavonoids	+	+	+	+
Gallic tannins	-	-	-	-
Polyphenolic compounds	+	+	+	+
Saponosides	-	+	-	-

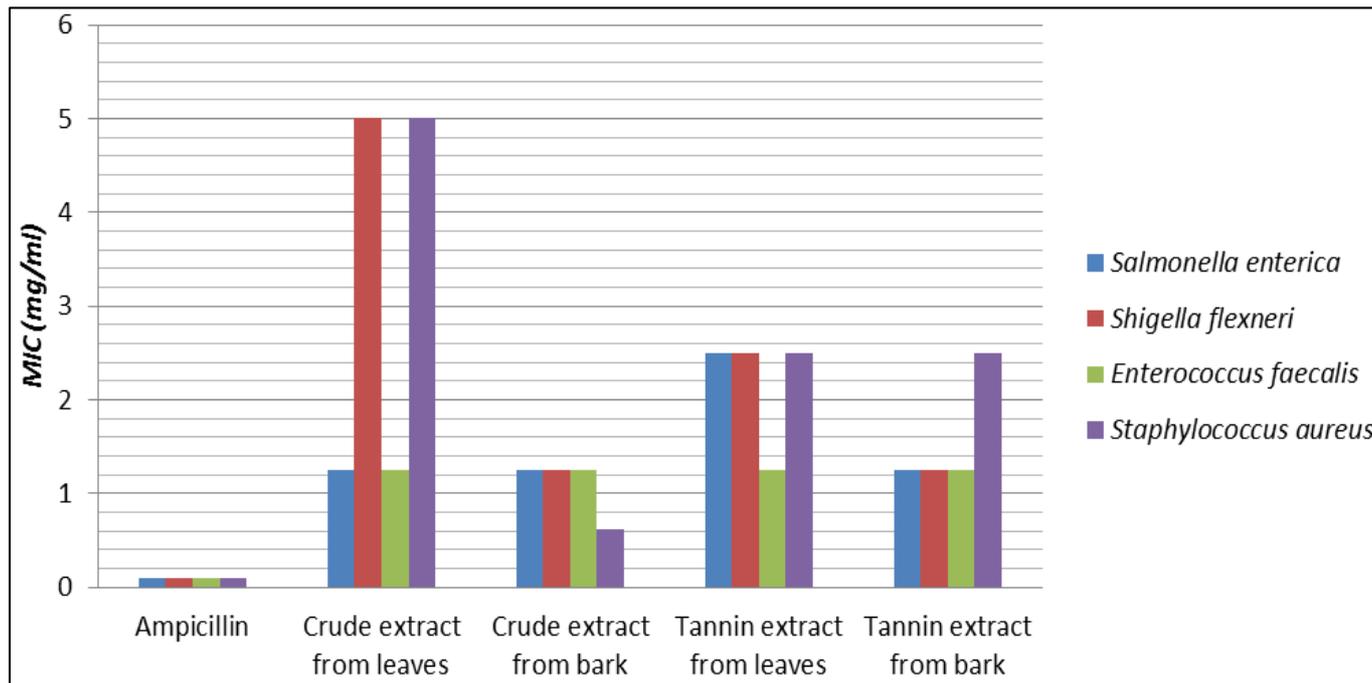
Legend: Positive test (+), Negative test (-), Crude extract from leaves (CEL), Crude extract from bark (CEB), Tannin extract from leaves (TEL), Tannin extract from bark (TEB).

The table 1 represents the preliminary phytochemical screening of different extracts of *C. anomala*. It was found, catechin tannins, flavonoids, and polyphenol compounds in

all extracts. The saponosides were present only on crude extracts. However, the absence of gallic tannins was noted in the all parts of the plant.

**3.2 Antimicrobial evaluations**

**3.2.1 Determination of minimum inhibitory concentrations (MIC)**

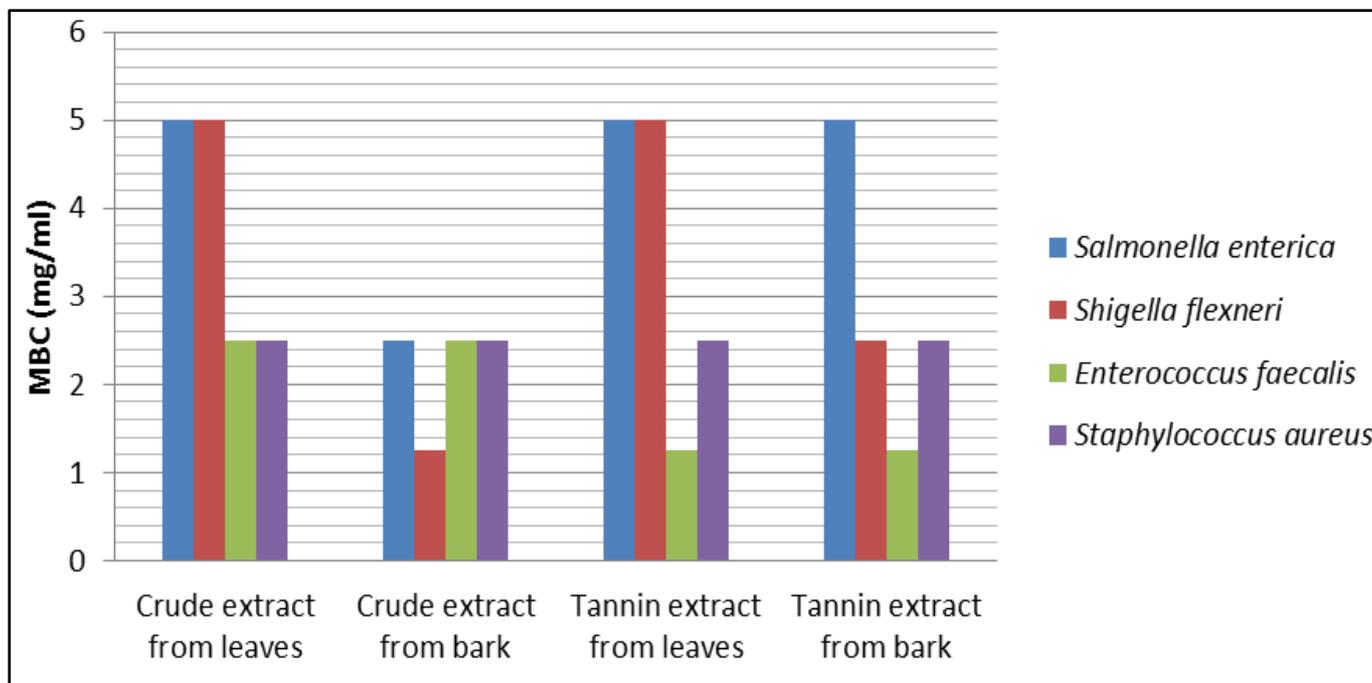


**Fig 1:** Values of minimum inhibition concentration (mg/ml) of reference and plant extracts.

Minimum inhibitory concentrations (MIC) of all extracts were between 0.625 mg/ml to 5 mg/ml respectively against *S. enterica* and *E. faecalis*. The Crude extract from bark was the most active with a MIC varied between 0.625 mg/ml to 1.25 mg/ml, while from leaves was the most inactive (CMI of 1.25

mg/ml to 5 mg/ml). For all strains, it was of 0.1 mg/ml for Ampicillin as reference.

**3.2.2 Determination of minimum bactericidal concentrations (MBC)**

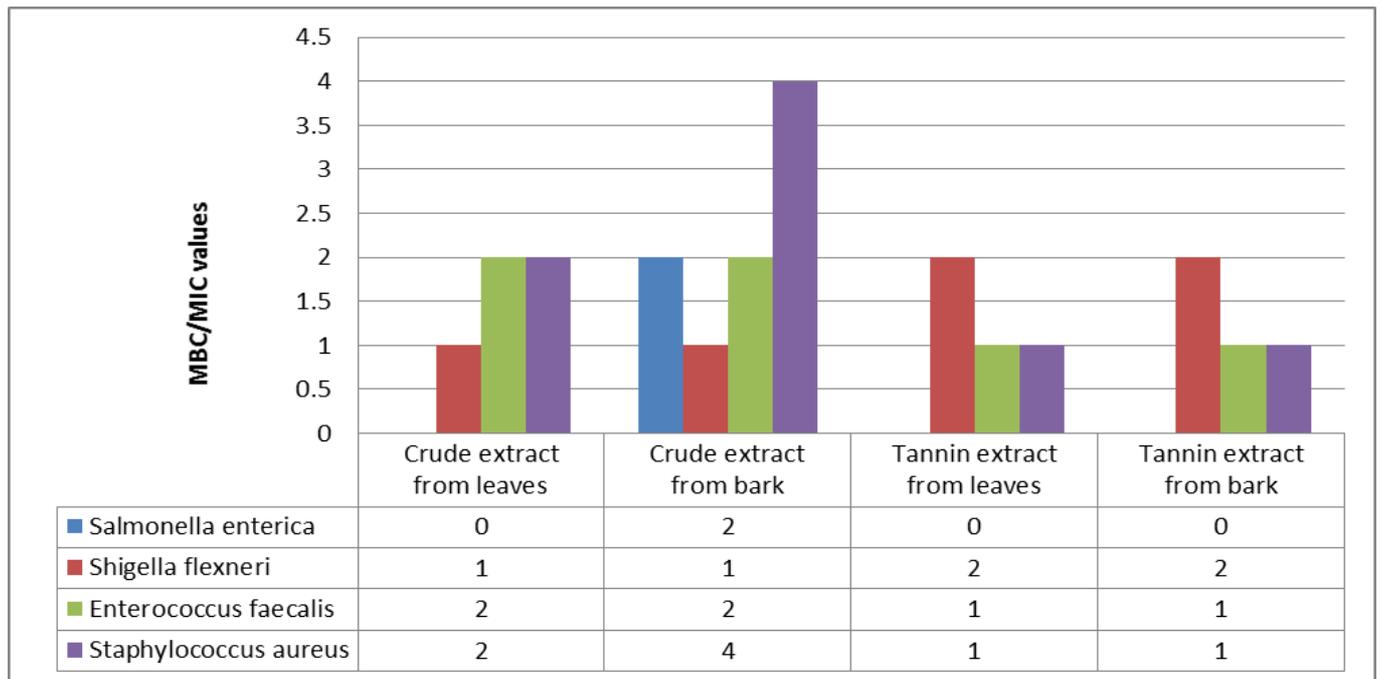


**Fig 2:** Values of minimum bactericidal concentration (mg/ml) plant extracts.

Values of MBC of plant extracts were determined on *S. flexneri*, *E. faecalis* and *S. aureus*. MBC values of extracts

varied from 1.25 to 5 mg/ml. For *S. enterica* only the MBC of crude extract from bark was determined.

### 3.2.3 Bactericidal effect of plant extract on strains



Legend: 0 (No determined)

**Fig 3:** Bactericidal effect of plant extract on strains

According to the results of Table 2, all plant extracts demonstrated a bactericidal activity against *S. aureus*, *E. faecalis* and *S. flexneri*. However, all extracts were not active (not bactericides) against *S. enterica* except the crude extract from bark.

#### 4. Discussion

Table 1 shows the result of preliminary screening of leaves and barks from *C. anomala* by usual methods based on color and precipitation reactions. The phytochemical investigations result indicated the presence of catechin tannins, flavonoids, and polyphenol compounds in all extracts. The saponosides were present only on crude extracts. A study on purine alkaloids and phenolic compounds in three *Cola* species grown in Cameroon, found the phenol patterns in *C. anomala* seeds. The sample from Baboate had the highest concentration (69.7 mg/g FW). More, the plant contained alkaloids (theobromine and caffeine) and flavonoids such as Catechin and epicatechin [15]. Antimicrobial evaluation was carried out on different strains mainly on *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella flexneri*. Ampicillin was taken as reference. According to the results from Figure 2, all extracts were active on *S. aureus*, *S. enterica*, *S. flexneri* and *E. faecalis*. They were inactive against *Pseudomonas aeruginosa* at the concentration of 5 mg/ml. In fact, this strain develops resistance through natural or antibacterial substances by production of beta lactamase enzymes. They can also develop an acquired resistance with several mechanisms [16].

Among all tested extracts (Figure 1 and Figure 2), crude extract of barks was most active ( $MIC \leq 1.25$  mg/ml). The high antimicrobial activity was with that extract against *Staphylococcus aureus* ( $MIC = 0.625$  mg/ml). That latter also had a weaker activity against *E. faecalis*, *S. enterica* and *S. flexneri* strains. However, the crude extract from leaves was the least active, with a  $MIC$  of 1.25 mg/ml against *S. enterica* and *E. faecalis*.

The tannin extract of barks showed a close  $MIC$  value compared to crude extract from leaves against *S. flexneri*, *S. enterica* and *E. faecalis* (1.25 mg/ml). However, the positive control with ampicillin presented the most activity with a  $MIC$  of 0.1 mg/ml against all strains compared to other groups. These  $MIC$  values were different from those reported by Agyare *et al.* (2012) [11] on *Cola gigantea*. Their  $MIC$  values on *S. aureus* were 0.250 and 0.125 mg/ml respectively for ethanol leaf extract and ethanol stem bark extract.

The tannin extract from leaves also showed a high activity compared to antimicrobial activity from crude extract. *E. faecalis* was the most sensitive ( $MIC$  of 1.25 mg/ml). These results are close to those obtained by Ngoupayo *et al.* (2016) [17]. According to their results, tannin extracts from *Erythrophleum guineensis* had a  $MIC$  of 1.25 mg/ml on *E. faecalis*. More, it was 2.5 mg/ml against *S. aureus*, *S. flexneri* and *S. enterica*. The tannin extract of leaves had a weaker  $MIC$  than that of crude extract against *S. enterica* and *S. flexneri*. In fact, tannins possess a good antibacterial activity. They are able to complex enzymes such as permease and porin. This complexation alters several vital functions of the bacterium such as respiration or synthesis of the components of the cell wall. Previous studies have shown that condensed tannins have the ability to inhibit beta lactamase production [17].

According to the results of Figure 3, the high bactericidal effect was obtained with tannic extracts against *E. faecalis* and *S. aureus* ( $MBC/MIC = 1$ ). The same results were found with crude extracts on *S. flexneri*. As far as this ratio was 2 for all tannic extracts against *S. flexneri* and crude extracts on *E. faecalis*. However, crude extract of leaves presented a bactericidal effect against *S. aureus* at  $MBC/MIC = 4$ .

The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds. A number of phytochemicals have been studied for their antimicrobial activity and found potentially useful against infectious diseases. The chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher

plants secondary metabolites such as flavonoids, terpenes, terpenoids, and phenolic acids<sup>[1]</sup>. Tannins inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism. Saponins have antibacterial activities thus have been used in the treatment of microbial infections<sup>18</sup>.

## 5. Conclusion

The result of this present investigation demonstrated the bactericidal potential of leaf and barks extracts from *C. anomala* on majority of tested strains. Therefore, further studies needs to be carried out on phytochemical and antimicrobial effect of compounds isolated from the plant materials.

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