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Characterization and evaluation *in vitro* of the antibacterial activity of tannins from *Garcinia brevipedicellata* (Bark.G) Hutch. & Dalz

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Abstract

The present study was conducted to evaluate the effectiveness of the tannic extract of *Garcinia brevipedicellata*, a plant used in Cameroon medicinal traditional, in treatment of bacterial infectious including food infections. The phytochemical characterization of tannins focused on the classic methods of screening. The macrodilution in liquid medium for 24 hours (acetone/water) allowed evaluating its antibacterial activity on the *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Vibrio cholera*, microbial strains. The phytochemical characterization was positive. The best inhibitory activity was obtained with *Salmonella Typhimurium* at 12.5mg/mL of MIC, with a ratio MIC/MBC of 2, and the best bactericidal activity on *Klebsiella pneumonia* at 50 mg/mL of MBC; with a ratio MBC/MIC \leq 4. The minimal inhibitory and bactericidal concentrations vary between 50 and 200 mg/mL. The present study revealed the antimicrobial potentials of tannic extract of *Garcinia brevipedicellata* on some microbial strains. A thorough study would be desirable for future use as a phytomedicine. Antibacterial activity, tannic extracts, minimal inhibitory and bactericidal concentrations, *Garcinia brevipedicellata*.

Keywords: *Garcinia brevipedicellata*, evaluation, antibacterial activity

1. Introduction

Infectious diseases still represent serious public health problems concern in terms of frequency and severity. These diseases are the leading cause of death in the world with nearly 17 million deaths each year [1]. In Cameroon, infectious diseases are among the most commonly reported and the largest cause of death [2]. Resistance to antimicrobial agents is becoming increasingly critical public health problem and such resistance to antibiotics is emerging in a wide variety of organisms and multidrug resistant organisms pose serious threat to the treatment of infectious diseases. Hence, plant derived antimicrobials have received considerable attention in recent years. It affects not only the economy, but the general well-being of people with more serious impacts in developing countries [3, 4]. *Staphylococcus aureus*, a typical example of bacteria involved in food poisoning causing gastroenteritis in people at risk, methicillin-resistant Enterobacteriaceae and extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* are among the most common resistant organisms [5]. Both invasive non Typhoidal *Salmonella Typhimurium* and *paratyphiare* important causes of invasive bacterial disease in Africa [6, 7]. Together with undesirable side effects and toxicity, the emergence of drug-resistant bacteria appears to be a major limitation of the use of antibiotics [8]. In addition, reducing the effectiveness of these synthetic drugs that arises as a result of the emergence of antimicrobial resistance and socio-economic context of African countries in general, making difficult the access of the population to pharmaceutical drug has prompted scientists to research on medicinal plants for their antimicrobial properties [9, 10, 11]. 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 [12, 13]. They have been used in different parts of the World to treat human diseases and infections [14]. The importance of medicinal plants as a source of new antimicrobials is well established today. About 25% of the prescriptions of active substances in the United States come from plant material [15]. The antimicrobial and biological activities of many plants have been attributed to various phytochemicals such as alkaloids, glycosides, tannins and saponins [16].

Many plants *Garcinia brevipedicellata* are used locally in Cameroon as traditional medicine for the treatment of infectious diseases [2]. It is part of the genus *Garcinia*, including ethnomedicine, different parts of its species have been reported to have many pharmacological effects [17]. It is part of the Clusiaceae family which is divided into two subfamilies. *Garcinia* contains about 260 species [18]. This study has been conducted to assess the effectiveness of antibacterial extracts of species of the genus *Garcinia* mainly *Garcinia brevipedicellata*.

2. Materials and Methods

2.1. Plant materials and preparation of extract

Stem barks of *G. brevipedicellata* were harvested in June 2014 at Mount Kala, 20 Km from Yaounde (Cameroon) and identified by Mr Nana Victor of Cameroon National Herbarium (CNH) where voucher specimen is deposited (HNC-23254). They were then pulverized by a mechanical grinder.



Fig 1: Dried bark of *Garcinia brevipedicellata*

The extraction procedure was performed according to the modified method [19]. The powder (2500 g) of *Garcinia brevipedicellata* was soaked at room temperature in 7500 mL of acetone (70: 30 v / v in water). During maceration, mixtures were stirred 3 times / day. It was made in two stages that is, during two successive days with change of solvent every 24h (7500 = 3750 x 2). The first macerate was filtered using Whatman® N°4. The resulting filtrate was collected; and then 3750 mL of solvent were added to the residue for a new extraction.

The two filtrates were combined into one single volume. The acetone was evaporated at low temperature to avoid thermal decomposition of the natural products. The residue was dried in an oven at 35 °C for 72 hours and pulverised in a mortar, then sieved and stored at 4 °C.

2.2. Chemical screening of tannins

The phytochemical analyzes were performed, focusing on the Stiasny reaction [20, 21]. The phytochemical screening summarized in the table below:

Table 1: Phytochemical characterization and indicator used

Secondary metabolit	Reagent used	Indicator
Polyphenols	Ferric chloride	Blue-black color
Catechuk tannin	Formalin (40%) Hydrochloric acid	Gelatinous precipitate
Gallic tannin	Lead acetate Ferric chloride	Blue-black color

2.3. Determination of antibacterial activity

2.3.1. Preparation of Bacterial Inocula

For each bacterial strain, few colonies of 18 to 24 hours of age were collected using wire loop. They were then introduced

into sterile saline, until turbidity similar to that of the point 0.5 of the McFarland scale was reached, corresponding to the concentration of 1.5×10^8 colony forming units / mL (CFU / mL). From this concentration, a dilution of 1/100 was made and used for the determination of MIC and MBC

2.3.2. Evaluation of the antimicrobial activity of extracts

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were the two parameters used to assess the antimicrobial activities of the studied plants. This activity was conducted on five clinical strains: *Escherichia coli* 00171, *Klebsiella pneumoniae* T81, *Salmonella Typhimurium* 4/14, *Staphylococcus aureus* 04 F118 op, and *Vibrio cholerae* O1

2.3.3. Determination of minimum inhibitory concentrations (MIC)

Macrodilution technique in liquid medium was used [22]; 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.

2.3.4. 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 [19]; and MBC determination was based on the subculture of bacterial inoculum from the MIC on nutrient agar [23].

In each of the tubes in which visible growth was not observed and the control tube used in determining the MIC [23], 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. Characterization of tannins

Table 2: Phytochemical characterization of tannins

Secondary metabolit	Reagent used	Conclusion
Polyphenols	Ferric chloride	+
Tannins	Lead acetate	+
Hydrolysable tannins		+
Catechuk tannins	Formalin (40%) Hydrochloric acid Ferric chloride Lead acetate	+

Key: +: Positive test

The minimum inhibitory concentrations (MIC), the minimum bactericidal concentrations (MBC), the ratio CMB / CMI (R) are shown in figure 2.

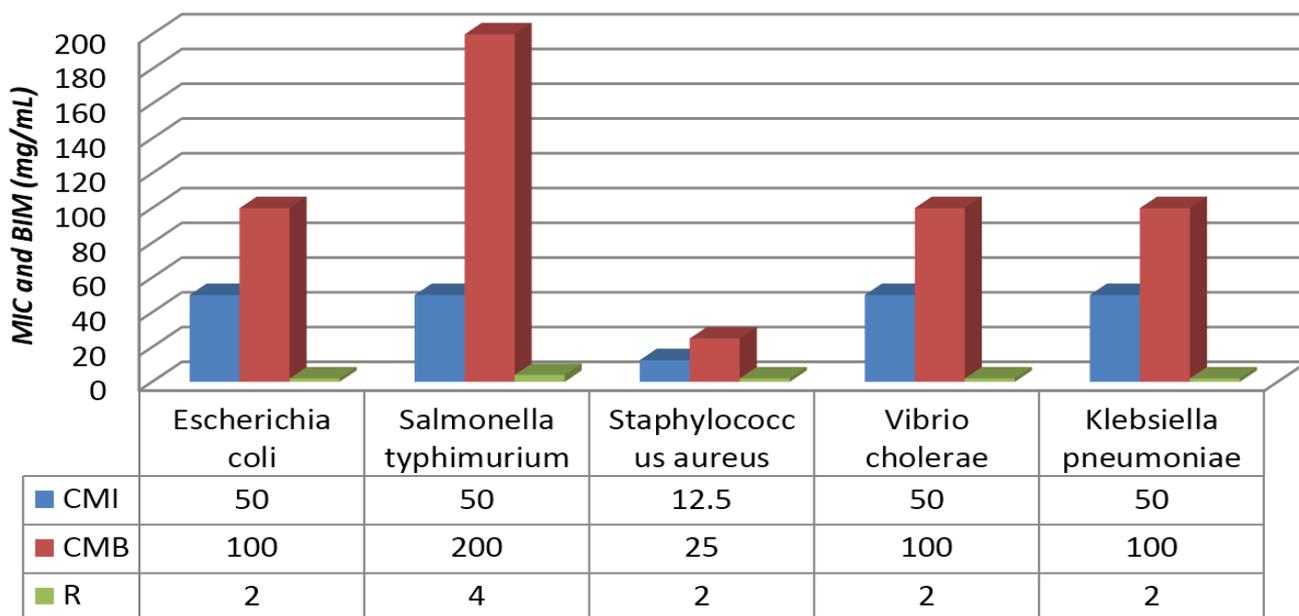


Fig 2: Evolution of minimum inhibitory concentrations (MIC) and bactericidal (MBC) and their ratio (R).
Legend: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration
 R: MBC/MIC.

The results in figure 2 revealed that our test plant extract recorded the smallest MIC and MBC values against *Salmonella Typhimurium* strain T88 4/14 (12.5 mg / mL and 25 mg / mL for MIC and MBC respectively). On the other hand, with the exception of *Klebsiella pneumoniae* strain T81 which could only be killed at MBC value of 200 mg/mL, *Garcinia brevipedicellata* exhibited similar antimicrobial activities (identical MIC and MBC values in the order of 50 mg/ml 100 mg/ml respectively) against the four remaining bacteria (*Escherichia coli* 00171, *Staphylococcus aureus* 04 F118 op, *Vibrio cholerae* 01)

4. Discussion

Previous phytochemical studies of *G. brevipedicellata* revealed a high presence of secondary metabolites including tannins [4]. In view of complementing what has also been known, we have in the present study also characterized their presence using various tests. Tannins which are polyphenol, gave a positive reaction to ferric chloride test; and the precipitate obtained from lead acetate indicated their presence in our extract. These two positive tests were confirmed by the Stiasny test which enabled us to characterise the different classes of tannins found in the bark of this plant. Tannins seem to be constant component of *Garcinia* species in general as the phytochemical screening has been demonstrating the presence of this compound in others varieties of this plant [4, 24, 25]. In fact, tannins have an antibacterial effect by precipitating their proteins making them unavailable their nutritional proteins [26]. Our plant extract recorded the smallest MIC and MBC values (12.5 mg / mL and 25 mg / mL for MIC and MBC respectively) on *Salmonella Typhimurium* T88 4/14 meaning that this *Salmonella* serovar was the most sensitive of the tested microorganisms. The sensitivity of *Salmonella Typhimurium* T88 4/14 to this plant extract is encouraging as this bacterium has been known to display multidrug resistance to common antibiotics [27]. Moreover this *Salmonella* serovar is

3.2. Evaluation in vitro of the antibacterial activity

The MIC, MBC and the ratio MBC/MIC recorded by the plant extract on the test bacteria are presented in the figure 2 below.

one of the non-typhoidal *Salmonellae* known to cause invasive bloodstream infection in children, the elderly and the immunocompromised people in Sub-Saharan Africa [6, 7]. However, better exploitation of this plant will be achieved if it also exhibit antibacterial activities against *Salmonella Typhi* and *S. Paratyphi* that exclusively cause typhoid fever in humans. On the other hand, this plant extract exhibits moderately similar antimicrobial activities against *Escherichia coli* 00171, *Staphylococcus aureus* 04 F118 op, *Vibrio cholerae* 01 (MIC and MBC values equal to 50 mg/ml and 100 mg/ml respectively) with the exception of *Klebsiella pneumoniae* T81). Which bactericidal response was high (200 mg/ml). 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, 28, 29, 30, 31]. The exceptionally high MBC value recorded on *Klebsiella pneumoniae* T81 may be attributed to its beta-lactamase activity that confers to this microorganism multidrug resistance property. This weak antibacterial property of this plant to the tested microorganisms differs from previous work carried out on the antibiological properties of other species of *Garcinia* [1, 24]. This difference may due to the chemical composition of the related cultivars of *Garcinia* or to the extraction method. The MBC/MIC ratio advocates for bactericidal effects of this plant extract but at a relatively high concentration (MBC value in order of 100 mg/ml). Despite its weak antibacterial property of *G. brevipedicellata*, its inhibitory effect on *Staphylococcus aureus* advocates for its broad spectrum antimicrobial properties against both Gram negative and positive organisms.

Conclusion

Phytochemical analysis revealed that tannins constitute the

secondary metabolites of the studied plant. The acetone extract of *G. brevipedicellata* had antibacterial against all the tested organisms, both Gram Negative and Positive bacteria though its inhibitory effect was relatively weak except against *Salmonella Typhimurium* 4/14. Other extraction methods should be carried out in future in view of enhancing its antimicrobial potentials. Moreover, this plant extract should also be tested against *Salmonella Typhi* and *S. Paratyphi* in view of exploitation for treatment of *Salmonella* infections. The mode of action of this plant activity was proved to be bactericidal on the basis of the MBC/MIC ratio.

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