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Antioxidant, anti-salmonella and anti-shigella activities of *Bambusa vulgaris* Schrad leaves. Ex JC Wendl. A medicinal plant used in Benin

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Abstract
Bambusa vulgaris is a world-famous medicinal plant used in Benin to treat typhoid fever, malaria, diarrhea, fatigue. The leaves have been investigated for their antioxidant, anti-shigella and anti-salmonella properties. Similarly, the phytochemical screening of ethyl and hydroethanolic extracts from the leaves was done according to the Harborne method with some modifications and revealed the presence of alkaloids, phenolic compounds, flavonoids, terpenenoids, tannins, anthraquinones, coumarin, and anthocyanins. Sterols, glucosides and saponosides are absent. The antioxidants tests for decocted extract was conducted by DPPH, ABTS and FRAP methods with respectively 406.4 µg/mL, 52.87 µg/mL and 103 µg/mL as scavenging concentration 50. These results confirmed the use of this plant as a natural source of antioxidant that could help in the fight against oxidative stress. The anti-shigella and anti-salmonella tests carried out by micro dilution method showed low activity (MIC > 2000 µg/mL) that can be explained by the method and the extraction solvent used.

Keywords: *Bambusa vulgaris, medicinal plant, Antioxidant, anti-salmonella and anti-shigella* activity

Introduction
Plants are the main source of food and treatment for humanity [1]. Approximately 1/3 of the currently marketed drugs contain at least one active plant substance, whether it be pest control, antibiotic, anticancer, immunosuppressants [2]. Thus the *Bambusa vulgaris* Schrad.Ex J.C. Wendl. of the Poaceae family, is a plant used both economically and pharmacologically. The rods are used in the manufacture of musical instruments, decoration and in the construction of houses. From a pharmacological point of view, the leaves of *Bambusa vulgaris* used in traditional medicine in the treatment of several diseases among which we have bacterial and parasitic diseases [3].
Indeed, this plant is known in the literature for its antidiabetic, antimicrobial, anti-lidemimic, anti-inflammatory, anti-obesity, anti-fatigue and anti-cancer activity [4]. Other pharmacological properties of *Bambusa vulgaris* are also tested, namely analgesic, antipyretic, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiviral, diuretic, hepatoprotective, abortifacient and anti-anxietic properties [5].
Apart from the identification of Alkaloids, tannins, coumarins, flavonoids and anthocyanins in leaf and root extracts of *Bambusa vulgaris* from Benin and the evaluation of toxicity [6], very few studies have been carried out on this plant in Benin.
The purpose of this work is to determine the antibacterial power more precisely those anti-salmonella and anti-shigella on the one hand and the antioxidant power of extracts of the leaves of *Bambusa vulgaris* on the other hand.

Material and methods
Plant material
The leaves of *Bambusa vulgaris* were harvested in March 2021 in the Atlantic department of Benin. The leaves of this plant were presented and identified in the Herbarium national of Benin under code YH760/HNB by Hounnankpon Yedomonhan. Then they were dried out from the sun and powdered.

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Biological material

The *in vitro* anti-salmonella and anti-shigella activities of the extracts was evaluated on six (6) bacterial isolates including *Salmonella enteritidis* (SE CPC); *Salmonella typhi* (ST CPC),*Salmonella typhimirium.* (STM CPC) and *Shigella dysenteriae* (SD CPC) from Centre Pasteur of Cameroon (CPC) and two (2) bacterial including *Shigella flexneri* (SFNR518) and *Shigella sonnei* (SONR519) from Bei resource. These strains were stored in the laboratory of Phytobiochemistry and Medicinal Plant Study/Antimicrobial and Biocontrol Agent Unit (AmBcAU) in tubes containing Muller Hinton agar by slant culture at 4EC.

Extraction

Three different extracts were obtained by decoction (aqueous extract) of each plant powder in water (50 g: 500 ml, w/v) for 45 mins at 90 °C and by maceration (ethanolic (95%) and hydroethanolic (70:30, V/V) extracts) of each plant powder in solvent (50 g: 500 ml, V/V) for 48h at room temperature. The mixtures were stirred twice a day and the macerates obtained were filtered using a wattman paper and then evaporated using a rotary evaporator (Buchi, 011) at 60EC. The process was repeated 3 times in order to deplete the plant material and the crude extracts were obtained and were stored in the refrigerator at a temperature of 4 °C.

Preparation of stock solutions of extracts and reference antibacterial

The stock solutions of the extracts (decocted, hydroethanolic and ethanolic) were prepared by dissolving 100 mg of each extracts in 1 mL of DMSO 10% (100 mg/ml). Ciprofloxacin used as a positive control was prepared under the same conditions at 1 mg/mL by dissolving 1 mg of powder in 1 mL of acidified distilled water.

Phytochemical screening

Two extracts (hydroethanolic and ethanolic extracts) were evaluated by phytochemical qualitative reactions for usual *Bambusa vulgaris* secondary metabolites. The screening was performed for anthocyanins, glucoside, terpenoids, sterols, alkaloids, anthraquinones, coumarins, flavonoids, saponosides, tannins, and phenolic compounds according to the protocol described by Habone (1998), with minor modifications. The ultimate goal of medicinal plant studies was often to isolate one or more constituents responsible for the particular activity of the plant [7].

Antisalmonel and antishigella activities of raw extracts:
Preparation of the bacterial suspensions

The different bacterial suspensions were prepared according to the 0.5 McFerland standard. For this purpose, a mother suspension was prepared at a turbidity of 0.5 McFerland ($1.5x10^8$ cells/mL) from 24-hour cultures on Muller Hinton Agar (MHA) and then diluted to $5x10^5$ cells/mL for testing.

Determination of minimum inhibitory concentration

The inhibition endpoint of the extract was evaluated by determining minimum inhibitory concentrations (MICs) using the liquid microdilution method described by CLSI [8]. The tests were performed in triplicates in sterile 96 wells microplates. Indeed, 160 µL of MHB culture medium were introduced into the first wells and 100 µL into the rest of the wells. Subsequently, 40 µL of extract solution (10 mg/ml) were taken and fed into the corresponding wells and followed by a series of 5 serial geometry dilutions of order 2. Finally, 100 µL of a bacterial suspension (5×10^5 CFU mL) were distributed in the test wells and those of negative control. The final Concentrations of extracts and ciprofloxacin in wells ranged from 1000 µg/mL to 31.125 µg/mL and 1.95 µg/mL to 0.0153 µg/mL, respectively. The percentage of DMSO in the first wells was 1% and showed no effect on bacterial growth. The microplates were coated and incubated at 37 °C for 24 hours. At the end of the incubation period, 10 µL of freshly prepared resazurin solution (0.15 mg/mL) was added to all wells and the plates were incubated again under the same conditions for 30 minutes. The lowest concentration at which there is no change in color from blue to pink corresponding to an absence of visible bacterial growth was considered MIC.

Trapping DPPH radical

The DPPH radical (2, 2-diphenyl-I-picryhydrazyl) is one of the most stable organic nitrogen radicals, with a purple color. It is a colorimetric method based on the loss of color at 517nm, proof of the reduction of the radical DPPH (2,2-diphenyl-1-picryhydrazyl). The protocol used for trapping the radical DPPH (2, 2-diphenyl-1-picrilhydrazyl) is that of Bassene with some modifications [9]. Briefly, 25 µL of extracts at different concentration (500;250; 125; 62.5; 31.25; 15.625 and 7.8125 µg/mL) and compounds (200;100; 50; 25; 12.5; 6.25 and 3.125 µg/mL) were added to 75 µL 0.02% DPPH solution (in methanol). The reading of the optical densities at 517 nm was made after 30 min incubation at ambient temperature and protected from light in the dark and at ambient temperature. Negative control consisted of 100 µL of DPPH solution without extract and ascorbic acid (100; 50; 25; 12.25; 6.25 and 3.78125 µg/mL) was used as positive control. The extracts were also tested alone under the same conditions to see which extracts are fluorescent. The tests were performed in triplicate. The antioxidant activity associated with the trapping effect of the DPPH radical is expressed as Trapping percentages (SC %) using the following formula:

$$SC\ \% = \frac{\text{Absorbance controle} - \text{Absorbance echantillion}}{\text{Absorbance controle}} \text{x } 100$$

Scavenging concentration 50 (SC_{50}) were calculated using the software GraphPad prism 5.0.

Trapping ABTS radicals

Antiradical activity was also evaluated by the $ABTS^{+}$• radical discoloration test using the protocol described by Khan *et al*. (2012) with some modifications [10]. 25 µL of each extract solution at different concentrations (500; 250; 125; 62.5; 31.25; 15.625 and 7.8125 µg/mL) were added to 75 µL of ABTS+ solution (7mM ABTS+4.9mM $K_2S_2O_8$ (1:1)). Then, the reading of the optical densities at 734 nm was made after 30 min incubation at room temperature and away from light. The negative control consisted of the ABTS reagent without extract and the positive control of ascorbic acid treated as extracts but with final concentrations of 100; 50; 25; 12.25; 6.25 and 3.78125 µg/mL. The extracts were also tested alone under the same conditions to see which extracts are fluorescent. The tests were performed in triplicate. The reduction in ABTS leads to a blue discoloration of the ABTS solution measured after 30 min incubation at 734 nm from the solution [11]. The lower the final absorbance, the higher the oxidant value the more effective the antioxidant [12].

The traping percentage (SC%) were obtained using the following formula:

$$SC\ (\%) = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \text{x } 100$$

The antioxidant activity associated with the trapping effect of the ABTS radical is expressed as Scavenging concentration (SC_{50}) using GrapPad prism 8.0.1 (124). SC_{50} (50% Scavenging concentration) is antiradical concentration needed to neutralize 50% of free radicals.

Results

Chemical results

Extraction yields with different solvents: The table below shows the extraction efficiency of *Bambusa vulgaris* leaves with different solvents.

Table 1: Extraction yields with different solvents

Plants	Part	Powder (g)	Ethanolic mass extracts(g) and yield %	Water/ethanolic (30 : 70) mass extracts(g) and yield %	Aqueous mass extracts(g) and yield %
Bambusa vulgaris	Leaves	50	3.1 (6.2)	5.0 (10)	5.3 (10.6)

The extraction yields of the leaves of *Bambusa vulgaris* with different solvents reveal that the best extraction solvent is water. Indeed, aqueous decoction has a yield of 10.6%.

Phytochemical screening

Phytochemical screening is carried out at the IMPM plant chemistry laboratory in Cameroon.

The results are given in Table 2

Table 2: Phytochemical screening of *Bambusa vulgaris* etanolic and hydroethanolic extracts

Chemical groups	Hydroethanolic extract	Ethanolic extract
Alkaloids	+	+
Phenolic Compounds	+++	+
Flavonoids	++	++
Terpenoidds	+	+
Sterols	-	-
Tannins	++	++
Glucosides	-	-
Anthraquinones (Quinones Bound)	+	+
Coumarins	+	+
Anthocyanins	+	++
Saponosides	-	-

Unidentified presence: Low + presence; Significant ++ presence; Very abundant: +++

Phytochemical screening of extracts revealed the presence of several large families' chemicals. Thus, this analysis showed the presence of alkaloids, phenolic compounds, flavonoids, terpenenoids, tannins, anthraquinones (quinones bound), coumarins and anthocyanins in the ethanol extract as in the hydroethanol extract of *Bambusa vulgaris* leaves. Similarly, the majority presence of phenolic compounds in the hydroethanolic extract than in the ethanolic extract showed the influence of the extraction by different solvents.

Antioxidant result

The aqueous, hydroethanolic and ethanolic extracts of *Bambusa vulgaris* have been tested for their antioxidant activity *in vitro*. The results are recorded in Table 3.

Table 3: Scavenging concentration 50 obtained by DPPH, ABTS and FRAP methods

Simples	DPPH	ABTS	FRAP
Extract	SC_{50}±SD (µg/mL)	SC_{50}±SD (µg/mL)	SC_{50}±SD (µg/mL)
BV1 aqueous	406.4±6.36	52,87±1.53	103±2.89
BV2 Water/ethanol	> 500	465.3±23.1	> 500
BV3 ethanolic extract	> 500	> 500	> 500
Acide ascorbic	7.36±0.31	22.46±2.73	33.6±1.01

According to Table 4, the results showed that the BV1 aqueous and Hydroethanolic extract had antiradcal properties on ABTS (SC50 = 52.87; 465.3 µg / ml, respectively).
Only decocted extract showed antioxidant properties on DPPH (SC_{50} = 406.40 µg/mL), ABTS radical (SC_{50}= 52.87 µg/mL) and FRAP (SC_{50} = 103.00 (µg/mL). All extracts with SC_{50} > 500 were considered as not possess antioxidant properties.

Biological activities

Antibacterial activity

Antibacterial results

The table below shows the anti-salmonella (table 4) and anti-shigella (table 5) result of ethanolic and hydroethanolic extracts from leaves *Bambusa vulgaris*.

Table 4: Minimum inhibitory concentration (µg/mL) on salmonella strains

Extracts		SE CPC	STM CPC	ST CPC
1	BV3 Ethanolic extract	> 2000	> 2000	> 2000
2	BV2 Water/ethanol	> 2000	> 2000	> 2000
3	BV1 aqueous extract	> 2000	> 2000	> 2000
Control	Ciprofloxacine	0,976	0,976	1,952

SE: *Salmonella enteritidis*, ST: *Salmonella typhi*, STM: *Salmonella typhimirium*, CPC: Pastor Centre of Cameroon.

Table 5: Minimum inhibitory concentration (µg/mL) on shigella strains

Extract		SFNR 518	SONR 519	SD CPC
1.	BV3 Ethanolic extract	> 2000	> 2000	> 2000
2.	BV2 Water/ethanol	> 2000	> 2000	> 2000
3.	BV1 aqueous extract	> 2000	> 2000	> 2000
Control	Amoxicilline	0.976	1.952	0.976

SFNR518: *Shigella flexneri*, SONR519: *Shigella sonnei*, SDCPC: *Shigella dysenteriae*, CPC: Pastor Centre of Cameroon.

All extracts from *Bambusa vulgaris* had not showed activity (MICs > 2000 g/mL) on both salmonella and shigella strains at the tested concentration (2000 g/mL).

Discussion

The analysis of the results of the extraction yields of *Bambusa vulgaris* leaves showed that the extraction yield varied according to the extraction solvents. Similarly, the best yield was obtained for aqueous decoction with a value of 10.6% against 10% for hydroethanolic and 6.2% ethanolic. Since water is more polar than the mixture water-ethanol, which in turn is more polar than ethanol. This difference in efficiency appears to be due to the influence of polarity on extraction efficiency. The same observation was made by Bero and *al.* during the extraction of *Trichilia emetica* [13]. The extraction efficiency of *Bambusa vulgaris* increases with the polarity of the solvent. It has been found that several factors influence

the efficiency of an extraction including the polarity of the extraction solvent [14].

Phytochemical screening of extracts revealed the presence of most of the secondary plant metabolites in ethanolic and hydroethanolic extracts. Qualitative analysis of secondary metabolites confirms the majority presence of alkaloids, phenolic compounds, flavonoids, terpenenoids, tannins, anthraquinones (quinones bound), coumarines, anthocyanins. Sterols, glucosides and saponosides could not be detected by our method. These results are consistent with those of Hessavi *et al*. [6] on the same plant harvested in Benin, and those of Owolabi and Lajide [15] in Nigeria with no phenolic compounds. The same results were obtained on the aqueous extract with saponosides in Côte d'Ivoire by Abe and his collaborators. It has been reported that the leaves contain quaternary bases and amines of saponides with absence of tannins [16]. This variation in constituents would appear to be due not only to the season, location, vegetative stage and time of harvest, but also to the extraction solvent.

The decocted, hydroethanolic and ethanolic extracts of *Bambusa vulgaris* leaves had not showed activities on both *Salmonella* and *Shigelles* strains (MICs > 2000 µg/mL.). These result can be explained by the geographical distribution of plants species and extraction procedure. Our results confirm with *Salmonella* those obtained by Ajobiewe his collaborators as the extract of leaves of the *Bambusa vulgaris*. Indeed, the anti-salmonella activity against *Salmonella tiphy* resulted in a minimum inhibitory concentration (MIC = 2500 µg /mL) [17]. Similarly, our results during the evaluation of anti-Shigella and anti-salmonella activity corroborate those obtained by Chung and Ko in 2005. Indeed, the latter evaluated these properties and showed that the sap of *Bambusa vulgaris* leaves inhibit *Salmonella typhi* and *Shigella dysenteriae* with inhibition diameters of 15 mm and 23 mm respectively. This justifies that the extracts of *Bambusa vulgaris* possess a considerable antibacterial power. The same observation was made by Menchavez *et al*., against *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* by Menchavez *et al*., [16] with inhibitions of 26 mm and 20 mm respectively of the ethanolic extract of leaves of *Bambusa vulgaris*. Other studies have shown that *Bambusa vulgaris* stem ethanolic extract has good activity against E. coli (8.64 mm to 8.86 12.54 mm inhibition) and S. aureus (13.75 mm to 12.54 mm inhibition) [18]. Nevertheless it has been reported by Owolabi and Lajide [17] in Nigeria that the leaves of *Bambusa vulgaris* have a good activated against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae.* In fact, it was shown that the hexanic extract, chloroformic and ethyl acetate inhibited Niger fungus (a minimum inhibitory concentration [MIC] = 1.25 ± 0.80; 2.5 ± 0.71 and 2.5 ± 0.71 mg/mL, respectively) and *Verticillium alboatrum* ([MIC] = 5.0± 0.71, 2.5± 0.71 and 1.25 0.55 mg/mL). The antibacterial screening of leaf extract against Gram-positive *Bacillus cereus bacteria* ([MIC] = 1.25 ± 0.23, 2.5 ±0.55 and 5.0 ±0.10 mg/mL), *Staphylococcus aureus* ([MIC] = 2.5 ± 0.77; 5.0 0± 0.10 and 1.25 mg/mL), and Gram-negative *Escherichia coli* ([CMI] = 2.5 ± 0.71; 3.5 ± 0.23 and 1.25 ± 0.23 mg/mL) and Klebsiella pneumoniae ([MIC] = 3.5 ± 0.23; 2.5 ± 0.10 et 1.25 ± 0.55 mg/mL) [16]. This difference in results could be explained by the difference in bacterial strains.

The obtained result with antioxidant test by three DPPH, ABTS and FRAP methods confirm those obtained by those who evaluated the antioxidant activity of ethanolic extract (300µg/mL) and hexaxanic extract (71µg/mL) with high antioxidant activity [19]. The aqueous extract of *Bambusa vulgaris* has a strong antioxidant power. The leaves of *Bambusa vulgaris* seem to be rich in natural antioxidants. It was reported by Tripathi *et al*. [20] when evaluating antioxidant activity using the DPPH method that *Bambusa vulgaris* would contain phenolic compounds that are responsible for the observed oxidizing power.

Conclusion

This research contributed to the phytochemical and biological study of *Bambusa vulgaris*. Phytochemical screening has revealed that the plant contains many secondary metabolites such as alkaloids, phenolic compounds, flavonoids, terpenenoids, tannins, anthraquinones (quinones bound), coumarin, and anthocyanins. The antioxidant test of the decocted extract shows that the leaves have a good antioxidant power that can fight oxidative stress. The species has low anti-salmonnella and anti-shigella activity with inhibitory concentrations (MICs>2000 µg/mL) against these strains. Nevertheless, it has been shown that *Bambusa vulgaris* has good activity against certain bacterial strains such as *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae*. *Bambusa vulgaris* would be an important source of natural antioxidant. To our knowledge, our results would be the first in relation to these activities studied for *Bambusa vulgaris* acclimatized in Benin. For our next work, we will study the pest control properties of the leaves of *Bambusa vulgaris*.

Conflict of Interest

The authors have not declared any conflict of interest.

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