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Phytochemical profile, antioxidant and antibacterial activities of *Cassia sieberiana* leaves from Benin and Niger specimens

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Abstract
Background: *Cassia sieberiana* is a plant often used in the treatment of various human diseases in Africa.: This is a comparative study of the phytochemistry and biological activity of leaves from samples collected in Benin and Niger.
Methodology: Phytochemical screening was performed using coloration and/or precipitation tests on the leaves. Total phenol content was determined using the method described by Wong *et al*., and total flavonoid content was determined using the method described by Agbangnan *et al*. DPPH radical reduction of the extracts was performed. Finally, microbiological tests on the extracts were based on the 96-well microdilution technique.
Results: Screening of the leaves revealed the presence of several secondary metabolites, with some differences between the two specimens. The total phenol and total flavonoid contents are much higher in the Niger specimen than in the Benin specimen. The extract from the leaves of the Niger sample, which has an IC_{50} = 2.044 mg/mL, is more active in reducing DPPH than that from the Benin sample, which has an IC_{50} = 3.4403 mg/mL. The Niger sample produced much lower minimum inhibitory concentrations on *Escherichia coli* ATCC25922 and *Vibrio cholerae* respectively (2.5 and 5 mg/mL).
Conclusion: The Niger sample is more active than the Benin sample. This could be explained by the high concentration of secondary metabolites.

Keywords: *Cassia sieberiana*, Benin, Niger, phytochemical, DPPH, microbiological test

Introduction
The history of plants is closely linked to that of humanity. Indeed, humans use plants in many areas: food, clothing, cosmetics, health, and even perfumery. The WHO estimates that 80% of the population of Africa uses plants for their health needs [1].
Of the 300,000 plant species identified on the planet, the World Health Organization (WHO) has listed 21, 000 plants used for medicinal purposes worldwide [2]. Plants are a new source of bioactive natural products whose ethnopharmacological effects have been demonstrated and used as a primary source for drug discovery Kumar *et al*., [3]. Currently, approximately 25% of pharmacological drugs are derived directly or indirectly from plants [4].
Cassia sieberiana DC is a small tree 7 to 15 m tall belonging to the Caesalpinioideae subfamily, which is part of the Fabaceae family, also known as legumes [5]. It is a remarkable shrub that has several traditional uses in Africa [6]. As a result, traditional medicine can be considered an integral part of primary health care, improving access to treatment. However, the active ingredients in plants, although toxic, are generally little known, mainly due to their complexity. With this in mind, the objective of this study is to determine the physicochemical characteristics and biological activities of hydro-ethanolic extracts from the leaves of *Cassia sieberiana* acclimatized in Benin and Niger.

Materials and Methods
Plant material
The plant material consists of leaves from *Cassia sieberiana* acclimatized in both countries (Benin and Niger); for the samples from Benin, harvesting was carried out in the commune of Tchaourou at coordinates 8° 53′ 00″ north, 2° 36′ 00″ east, located in the department of Borgou; and for the samples from Niger, the harvest was carried out in the commune of Aguié,

bounded by coordinates 07.56°-07.85° east longitude and 13.23°-13.74° north latitude. It is located in the southern part of the Maradi region, 80 km along National Road No. 1 (RN1). For the study, *C. sieberiana* leaves were collected, dried, pulverized, and stored under laboratory conditions (27°C, protected from light) until their subsequent use. Taxonomic identification is carried out by the relevant institutions (National Herbarium of the University of Abomey Calavi in Benin and Abdou Moumouni University in Niger).

Phytochimical Screening

Phytochemical screening based on coloration and/or precipitation tests was performed on the bark of *Cassia sieberiana* stems from Benin and Niger. Alkaloids, coumarins, and saponosides were identified using the method described by Agbangnan *et al*. [7]. Anthraquinones, tannins, and flavonoids were detected using the methods described by Ahouansou *et al*.,: Thiaw *et al*., [8, 9].

Extraction

Powder from the leaves of *Cassia sieberiana* stems from both countries was used to prepare the extracts. Three hundred grams of powder were macerated for three days in 3 liters of hydroethanolic solvent in a ratio of 30/70.

Determination of phenolic compounds

Total phenols were determined using the Folin-Ciocalteu colorimetric reagent according to the method described by Agbangnan *et al*.,: Wong *et al*., [10, 11] The quantification of total phenols was determined using the linear calibration curve produced from a standard solution of gallic acid at different concentrations.

Total flavonoid content

The total flavonoids contained in the tested extract were measured using aluminum trichloride according to the method described by Enujiugha, *et al*., [12]. The flavonoids were quantified based on a linear calibration curve produced using a quercetin standard solution at different concentrations.

Determination of anti-radical activity

The anti-radical activity of the extract was evaluated using the spectrophotometric method described by Lagnika *et al*., [13]. Six (6) concentrations ranging from 0.25 mg/mL to 0.0078 mg/mL of extract prepared in methanol were tested. A 2% solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) diluted in methanol was used as the free radical. The absorbances were read at 517 nm and ascorbic acid was taken as the reference with the same concentration ranges. The percentage inhibition (PI) of DPPH (I%) was calculated using the following formula.

$$\%PI = \frac{A_{blanc} - A_{échantillon}}{A_{blanc}}$$

Antibacterial activity tests

The microdilution method on a 96-well microplate is used with iodonitrotetrazolium (INT) as an indicator of bacterial growth.

Preparation of the bacterial suspension

The four bacterial strains *Escherichia coli* ATCC 25922, *Escherichia coli*, *Salmonella sp*, and *Vibrio cholerae* are seeded on a solid culture medium. Suspensions of well-isolated bacterial colonies are introduced into a nutrient broth (MH). The bacterial suspensions obtained after incubation were adjusted to 10^6 CFU (McFarland standard), corresponding to an optical density OD = 0.001 at λ = 620 nm [14]. The antibacterial activity was evaluated by testing the sensitivity of bacterial strains to extracts at 10 mg/mL and determining the minimum inhibitory concentrations of the extracts.

Susceptibility testing of bacteria to extracts

The purpose of this test is to determine the efficacy of the extracts on bacterial strains at a concentration of 10 mg/mL. To do this, 100 µL of bacterial inoculum is added to 100 µL of extract concentrated at 20 mg/mL. After incubation at 37°C for eighteen (18) hours, 40 µL of an aqueous solution of INT concentrated at 0.2 mg/mL is added. The appearance of a pink or red color in the wells where bacterial growth has occurred indicates that the bacteria are insensitive to the extracts.

Determination of minimum inhibitory concentrations

Concentration ranges of 10 mg/mL; 5 mg/mL; 2.5 mg/mL; 1.25 mg/mL; 0.625 mg/mL; 0.3125 mg/mL; 0.15625 mg/mL were prepared for the extracts. 100 µL of MH broth were added to all wells of a 96-well microplate, then 100 µL of 20 mg/mL extract stock solutions were added to the first wells, from which successive half dilutions were made to the last wells. Next, 100 µL of bacterial broth concentrated at 0.001.106 CFU/mL were added to all wells. After eighteen (18) hours of incubation at 37°C, bacterial growth was verified by INT staining.

Results and Discussion

Phytochemical screening

Phytochemical screening of *Cassia sieberiana* leaves from Benin and Niger revealed the secondary metabolites listed in Table 1.

Table 1: Secondary metabolites identified

Part used Chemical groups	CSFB	CSFN
Alkaloids	+	-
Tannins	+	+
Catechin tannins	-	+
Gallic tannins	-	-
Flavonoids	+	+
Anthocyanins	+	+
Leucoanthocyanins	-	+
Saponins	+	+
Mucilages	-	-
Reducing compounds	-	+
Coumarins	+	+
Anthraquinones	+	+
Quinones	-	-

Legend: Presence (+): absence (-): Leaves of *Cassia sieberiana* from Benin (CSFB); Leaves of *Cassia sieberiana* from Niger (CSFN).

Comparison of the results for the two specimens from Benin and Niger reveals the presence of flavonoids, tannins, anthocyanins, saponosides, coumarins, and anthraquinones in both specimens, while quinones and mucilages are absent. Leucoanthocyanins and reducing compounds are identified in the specimen from Niger. Alkaloids were found only in the Benin specimen. These results are consistent with those of Evenanmede *et al*., who showed that the three organs of the plant contain major chemical groups such as flavonoids, tannins, and saponosides. [15]. They are also similar to those obtained by Awomukwu *et al*., who reported the presence of

alkaloids, flavonoids, saponins, and tannins in four organs of the plant: leaves, stems, roots, and pods [16]. The differences in chemical composition observed in the two specimens from Benin and Niger could be due to the nature of the soil, the geographical environment of the plant, and the harvest period.

Total phenol content: The total phenol content in the different samples is expressed in mg gallic acid equivalent per gram of extract (mg GAE/g). The values for total phenolic compound content vary between 56.24 and 72.6 mg GAE/g of extract; the results are shown in Figure 1.

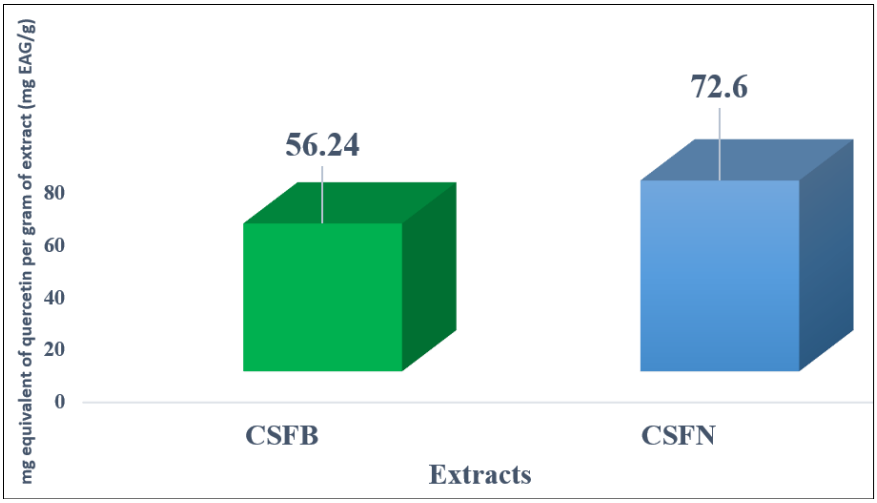

Fig 1: Total phenol content

Legend: Leaves of *Cassia sieberiana* from Benin (CSFB); Leaves of *Cassia sieberiana* from Niger (CSFN).

Comparison of samples from both countries with those from other studies shows that the phenol contents detected are significantly higher than those reported for the ethanolic extract from Togo by Evenamede *et al*., [15]. However, they are significantly lower than the values found by Jibril *et al*. in roots using solvents such as hexane, ethyl acetate, water, and butanol [17]. These differences could be due to soil type, environmental factors affecting the plant, and extraction solvents.

Total flavonoid content

The flavonoid content in the different samples is expressed in mg quercetin equivalent per gram of extract (mg QE/g). These values range from 189.78 to 512.82 mg QE/g of extract. The results are illustrated in Figure 2.

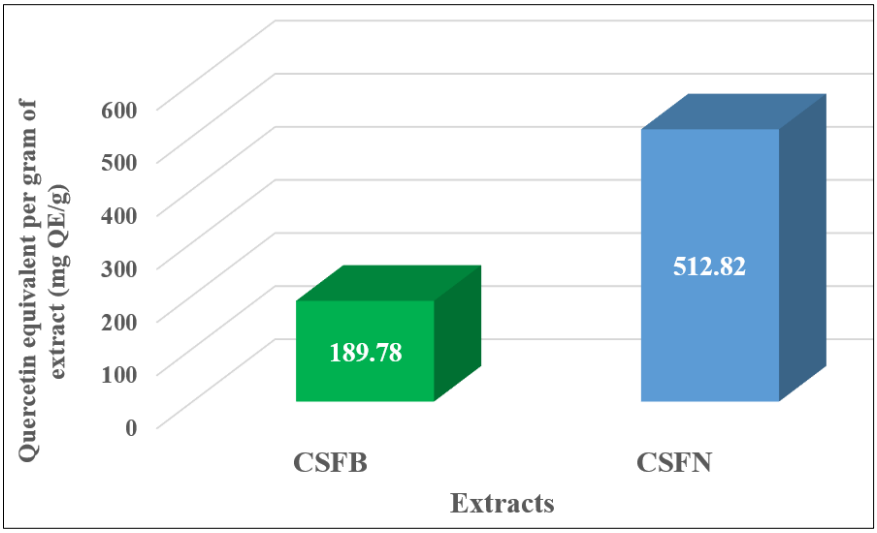

Fig 2: Total flavonoid content of the extracts tested

Legend: Leaves of *Cassia sieberiana* from Benin (CSFB); Leaves of *Cassia sieberiana* from Niger (CSFN).

The highest concentration of total flavonoids was observed in the Niger sample (512.82 mg EqQ/g of extract). This content is significantly higher than those observed by Jibril *et al*. However, in samples from Benin, the total flavonoid concentration recorded in the leaves (CSFB) is 189.78 mg EQ/g of extract, comparable to those found [17]. It should be noted that the total flavonoid content of the samples from Niger is significantly higher than that of the samples from Benin. Our levels are significantly higher than those found by Evenamede *et al*., [15].

Anti-radical activity tests

The reduction of the DPPH radical is dose-dependent; the higher the concentration, the higher the percentage of inhibition. The results, expressed as a percentage reduction in DPPH, vary between 7.74% and 94.64% for the different extract concentrations 78.10^{-4} and 2500.10^{-4} (mg/mL).

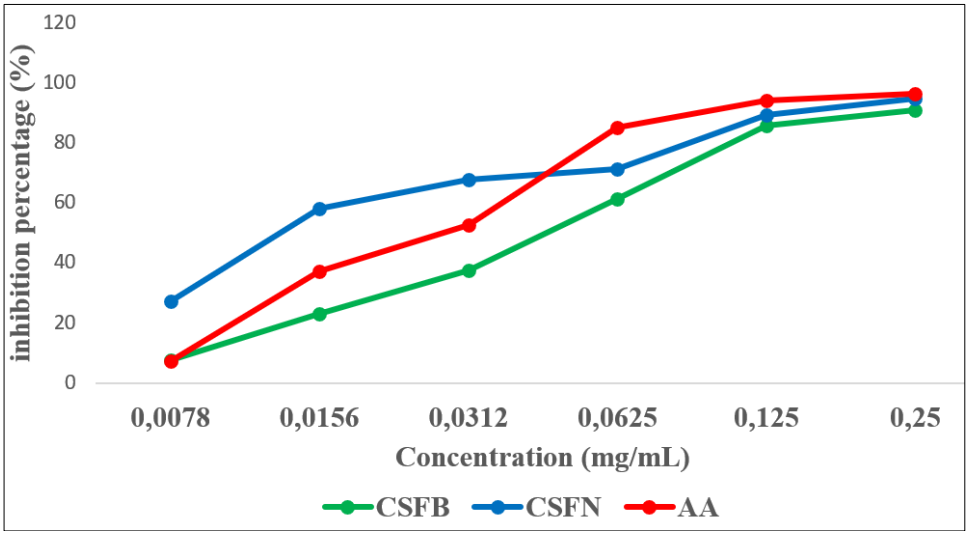

Fig 3: DPPH radical inhibition as a function of extract concentrations

Legend: Leaves of *Cassia sieberiana* from Benin (CSFB); Leaves of *Cassia sieberiana* from Niger (CSFN); Ascorbic acid (AA).

Determination of IC_{50} values: The IC_{50} value is calculated for each extract (Figure 6). The lower the IC_{50} value, the more antioxidant the extract is. It was calculated from the linear trend curves for each extract, and the values range from 2.044 to 3.4403 mg/mL.

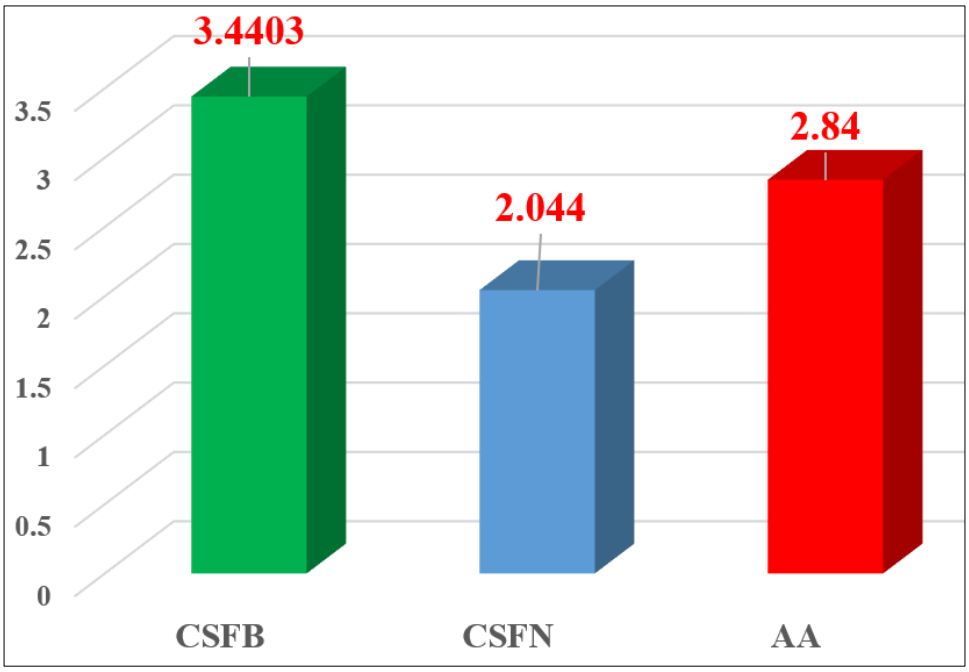

Fig 4: IC_{50} of the different extracts

Legend: Leaves of *Cassia sieberiana* from Benin (CSFB); Leaves of *Cassia sieberiana* from Niger (CSFN); Ascorbic acid (AA).

The anti-radical activities of the extracts from the various samples from Benin and Niger are compared to that of ascorbic acid, taken as a reference in terms of equivalence and inhibition. The strongest anti-radical activity is observed in the Niger specimen (IC50 = 2.044 mg/mL), whose IC50 is lower than that of ascorbic acid (2.84 mg/mL). In contrast, for the Benin samples, the DPPH radical inhibition of the leaves (CSFB) has an IC50 of 3.4403 mg/mL. Our results are similar to those found by Halilu *et al*., 2017; Traore *et al*., 2015 [18, 19]. They are significantly higher than those found by [17, 20, 21] in roots using different solvents of increasing polarity in leaves and roots, respectively.

Determination of Minimum Inhibitory Concentrations on bacterial strains

This is the lowest concentration of plant extract or antibiotic that can inhibit all visible growth of a bacterial strain after 18 hours of culture at 37°C.

Table 2: Different Minimum Inhibitory Concentrations

	E. coli	*E. coli* ATTC 25922	*Salmonella sp*	*Vibrio cholerae*
CSFB	ND	5	10	ND
CSFN	ND	2, 5	10	5

ND = not determined; *Cassia sieberiana* leaves from Benin (CSFB); *Cassia sieberiana* leaves from Niger (CSFN).

Analysis of the results in Table 2 shows the different minimum inhibitory concentrations of the extracts on the four

bacterial strains tested. The minimum inhibitory concentration is inversely proportional to the activity of the extract; the lower the MIC, the greater the activity. However, the extract from *Cassia sieberiana* leaves from Benin (CSFB) had minimum inhibitory concentrations (MIC) of 5 mg/mL and 10 mg/mL, respectively, on the *Escherichia coli* ATTC 25922 and *Salmonella sp*. strains. However, no MIC was determined for *Vibrio cholerae* and *Escherichia coli* at the doses tested. These results are similar to those found by L. Traoré *et al*., 2015 [22] in aglycone extracts from the bark of *Cassia sieberiana* roots. In the Niger specimen, the lowest minimum inhibitory concentrations (MIC) were determined for the Escherichia coli ATTC 25922 strain at 2.5 mg/mL. Next, for the *Vibrio cholerae* strain, MICs of 5 mg/mL were recorded. Finally, for the *Salmonella sp* strain, an MIC of 10 mg/mL was determined. The differences observed are due to the distribution of secondary metabolites in specialized tissues and only at certain stages of plant development. Our results are similar to those found by Halilu *et al*., 2017; Kpegba *et al*., 2019 [18, 23]. It is noted that the extract from the Niger specimen is more active than that from the Benin sample. This could be explained by the high concentration of secondary metabolites with antibacterial activity in the Niger samples and a low concentration in the Benin samples.

Conclusion

This study enabled phytochemical screening, phenolic compound analysis, and anti-radical and antibacterial testing of *Cassia sieberiana* leaves acclimatized in Benin and Niger. The Niger sample is more active than the Benin sample, which is justified by the high concentration of secondary metabolites. The anti-radical potential evaluated for the two extracts from *Cassia sieberiana* leaves shows that all extracts revealed DPPH scavenging power. Indeed, the highest capacity was observed in the Niger extract. Antibacterial power was observed much more in the extract from Niger leaves on the four bacterial strains tested.

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