Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(2): 44-47
Received: 21-01-2023
Accepted: 24-02-2023

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Phytochemical screening, phenolic determination and antibacterial activity of the extracts of *Bridelia scleroneura* Muell. Arg. (Euphorbiaceae) from Chad

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DOI: https://doi.org/10.22271/phyto.2023.v12.i2a.14629

Abstract
Bridelia scleroneura Müll. Arg. is traditionally used in the southern part of Chad to treat diabete, hypertension and anemia. This study reports the chemical composition of *B. scleroneura* leaves, barks twigs extracts and their polyphenols contents and also their antibacterial activity. Phytochemicals were assessed by phytochemical screening and spectrophotometry. The microdilution method was used to evaluate the antibacterial activity of extracts against *Staphylococcus aureus* (ATCC25923 /ATCC43300), *Streptococcoccus pneumonia* (ATCC461916), *Pseudomonas aeruginosa* (HM801), *Klebsiella pneumonia* (clinical isolate), *Salmonella typhi* (clinical isolate) and *Eschierichia coli* (clinical isolate). The extracts showed the presence of alkaloids, saponins, terpenes, steroids, polyphenols, tannins, quinones and flavonoids. The flavonoid content is higher in the leaves, tannin and polyphenol contents are higher in the barks. The antibacterial tests indicated that the bark extract exhibited a significant activity on *Staphylococcus aureus*.

Keywords: *Bridelia scleroneura*, polyphenols, antibacterial, *Staphylococcus aureus*

Introduction
Bridelia scleroneura Müll. Arg. is a savannah shrub or tree to 15 ft or more, widely distributed and commonly growing from Guinea to Sudan and in of Eastern Africa [1]. In the subtropical and tropical area of the world, especially in Africa and Asia, around 60 different species of Bridelia are distributed [2]. Some of these species are used in folk medicine to treat diseases of viral and microbial origin, some others are considered as laxative, hypoglycemic and antianemic [2]. In Chad, *Bridelia scleroneura* is a tropical herb common in South vegetation of Chad with extensive medicinal applications. The plant is locally called 'Chie Bian' in Ngambaye dialect [22]. The decoction of roots is used against diabete and hypertension while that of the trunk bark is used to treat anemia [22].
Previous analytical reports in genus *Bridelia* exhibited the presence of flavonoids, quinines alkaloids, saponines, tannins and phytosterols [17, 19, 20].
The present work was aimed at determining qualitatively the bio compounds as well as phenolics in different parts of *Bridelia scleroneura.* Therefore, because of the relationship between *Heliocobacter pylori* infection and anemia previously reported [23], the antibacterial activity of the extracts was performed.

Materials and Methods
Collection of plant samples
The leaves, trunk bark, and twigs of *Bridelia scleroneura* were collected on February, 2021 in the village of Bedogo (southern part of Chad). The plant was identified by comparing the specimen with the voucher samples kept in the Herbarium of the Institute of Breeding Research for the Development, N'Djamena, Chad.

Extraction
Samples were separately dried at room temperature and then grounded into powders. About 30 g of each part was macerated in 300 mL of solvent for 24 hours at room temperature. Solvents used for extraction were methanol, ethanol and ethanol/water (7:3, v/v).

After filtration on filter paper, the residue was extracted twice using the same procedure. The filtrates were evaporated to dryness using a rotary evaporator (BUCHI 461 type) under controlled temperature and pressure. The obtained crude extracts were kept in sealed vials and stored in the fridge for future work.

Phytochemical screening

Plant extracts were investigated in order to identify secondary metabolites such as flavonoids, alkaloids, tannins, saponins, quinones, sterols, terpens and polyphenols. The methods used to carry out these assays were those described by Wadood *et al*. and Harboune [11, 12].

Determination of total phenolics content

The determination of total phenolic content was performed according to the Folin-Ciocalteu method [24, 25]. In fact, 0.5 mL of the diluted extract (1 mg/mL) was mixed to 2.5 mL of the reagent of dilute Folin-Ciocalteu 0.2 N (1:10 dilution) and then shaken and left to stand for five minutes at room temperature to allow for the reagent to react completely with the oxidible substances or phenolates. 2 mL of Na_2CO_3 (5% in water) were added to destroy the residual reagent. The measurements of absorbances were performed with a spectrophotometer (Termoscientific Evolution 300) at 760 nm, after incubation for 2 hours in a darkness against a blank (distilled water). The calibration curve equation (y = 0.0088x - 0.0067, R^2 = 0.9926) was used to determine the total phenolic contents of the samples. They were expressed in mg Gallic Acid Equivalents (GAE)/100 g of dry material [4]. All measurements were duplicated.

$$C = \frac{C1 \times V}{m}$$

Where C expressed in mg equivalent gallic acid/g of dry material is the content of total polyphenols, C1 expressed in mg/L is the concentration of gallic acid derived from the calibration curve, V expressed in L, is the volume of extract and m expressed in g, is the weight of the plant extract [24, 25].

Determination of total flavonoids content

The determination of total flavonoid content was done by the method described by Arvouet – Grand *et al*. in 1994 [5]. A volume of aluminum chloride in methanol (2 mL, 2%) was mixed with the methanolic solution of the extract (2 mL, 1mg/mL). After 10 min incubation, the measurements of absorbances were done at 415 nm. The calibration curve equation (y = 0.0281x + 0.0052. R^2 = 0.9922) was used to determine the total flavonoid content. The previous formula used for the total phenolic content is the same used here and expressed in mg of Quercetin Equivalents (QE)/100g dry material [6]. All measurements were duplicated.

Determination of total tannins content

Tannin was determined using the reaction described by Bate-Smith [7]. In fact, 2 mL of each plant extract (1mg/mL) was introduced in a hydrolysis tube and 3 mL of hydrochloric acid (37%) was added. The closed glass tube was heated at 100°C in a water bath for 30 minutes after which the measurement of the optical density was done at 550 nm. The control was done with the same solution kept in a tube and left at room temperature. The following formula allowed to calculate the total tannin contents:

C = 19.33(Doh – Dot), where C expressed in g/L is the total tannin content, Doh is the optical density of the hydrolyzed tube and Dot is the optical density of the control tube [8, 9, 24, 25].

Antibacterial assay

Microbial strains

The microorganisms used were: *Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC43300, *Steptococcus pneumoniae* ATCC461916, *Pseudomonas aeruginosa* HM801, *Klebsiella pneumonia* clinical isolate, *Salmonella typhi* clinical isolate and *Eschierichia coli* ATCC25322.

Plate dilution tests

The antibacterial activity of crude extracts was determined using Agar plate dilution method provided by CLSI in the Protocol M07-A9 [10]. For the assay, a suspension was prepared at turdibidity 0.5 McFarland (corresponding to an approximate concentration of $1.5x10^8$ cells/mL) from cultures of 24 hours on Muller Hinton Agar (MHA) and then diluted to $5x10^5$. The different bacterial suspensions were prepared according to the 0.5 Mc Farland standard. The solutions of extracts were prepared at 10mg/mL of DMSO 10%. Gentamicin prepared at 1mg/mL in acidified distilled water was used as positive control.

Muller Hinton Broth dilution test

Muller Hinton Broth (MBH) dilution method was used to determine the minimal inhibition concentration (MIC) values of extracts on the sensitive bacterial strains. Into the first wells, an aliquot of 160 μL of culture medium were placed and 100 μL into the rest of the wells. Subsequently, 40 μL of a sterile solution of extract at 10 mg/mL were placed into the corresponding wells and followed by 5 serial dilutions by dividing the concentration by 2 at each dilution. Finally, 100 μL of a bacterial suspension at the load of 10^6 cells/mL were distributed in the test wells and those in the negative control. Concentrations of extracts and Gentamicin in wells ranged from 1000μg/mL to 31.125 μg/mL, and from 1.95 μg/mL to 0.0153 µg/mL respectively. The final charge of the inoculum in each well was $5x10^5$cells/ml. The microplates were covered and then submitted to incubation at 37°C for a period of 24 hours. After the incubation period, 10 μL of a resazurin solution freshly prepared (0.15 mg/mL) was added to all wells and the plates were once again submitted to incubation under the same conditions for a period of 30 minutes. The MIC is defined as the lowest concentration of extract where no change is observed in coloration from blue to pink, that point corresponding to a lack of visible bacterial growth [10]. The tests were duplicated in the sterile 96 wells microplates.

Results and discussion

Chemical contents

The result of the phytochemical screening of aqueous extracts of barks, twigs and methanolic extracts of leaves of *B. scleroneura* is showed in Table 1. The presence of these phytoconstituents in the different parts of the plant suggested many pharmacological activities which supported its traditional use in folk medicine. The presence of saponines and tannins was therefore responsible for the antibacterial activity of this plant. This is in agreement with the works reported by Yunana *et al*., in 2018 [18]. Since polyphenols exhibited an anti-inflammatory, antimicrobial and antioxidant effects [21] and they were present in all extracts, they could be

the source of the anti-nociceptive and anti-inflammatory effect of *Bridelia scleroneura* that described by Dimo *et al*., in 2006 [3].

Table 1: Results of preliminary phytochemical screening

Tests Samples	Alk		Tan		Flv	Sap	Quin	Ter/str	Poly
	D	M	Cat	Gal					
Twig extract	+	+	-	+	+	+	+	+	+
Bark extract	+	+	+	-	+	+	+	+	+
Leaf extract	+	-	+	+	+	+	+	+	+

Legend:Alk= Alkaloids; Tan= Tannins; Flv = Flavonoids; Sap = Saponosids; Quin = Quinones; Ter/str= Terpens/Sterols; Poly = Polyphenols; Cat: Catechic, Gal: GallicAbsent = -; Present = +; D= Dragendorff reagent; M= Mayer reagent.

Total phenolic, flavonoid and tannin contents

Results in Table 2 showed that the total phenolic content of the methanolic extract of the leaves, twigs and barks of *Bridelia scleroneura* ranged from 171.0±1.59 to 226.3±0.57 mg GAE/g of dry extract. The highest total phenolic content was observed in the barks extract and the twigs had the lowest value. The highest total flavonoid content (31.1±0.03QE/ 100 mg) was observed in the leaves extract whereas the barks extract had the lowest value (0.71±0.03 QE/ 100 mg). The total tannin content ranged from 11.46±0.29 to 53.34±0.24 g/L. The highest value of total tannin content was from the bark extract and the lowest value was from the leaves extract.

According to the results, the barks of *B. scleroneura* were rich in polyphenols (226.3±0.57 mg GAE/g of dry extract) and tannins (53.34±0.24 g/L) (Table 2). This is quite important than those obtained from the pericarp of the fruit of *B. stipularis* [13] but almost similar to those obtained from the stem bark of *B. speciosa* [14]. Phenolic contents from the leaves of *B. scleroneura* were twice less important than those reported by Cumbane and Munyemana in 2017 from the leaves of *B. cathartica* (427.53±10.41 mg GAE/g of dry extract) [21]. Based on these results, the leaves of *B. scleroneura* could be considered as a source of storage of phenolic compounds. The plant has the low flavonoid content, the highest one was found from its leaves (31.1±0.03 mg QE/g of dry extract), which was less important compared with the results obtained in the fruit pericarp acetone extract of *B. stipularis* (44.67 mg QE/g dry mass) but higher than the one obtained in the seed ethanol extract of the same plant (7.17 mg QE/g dry mass) [13].

Table 2: Results of totat phenolic, flavonoid and tannin contents

Samples	Total polyphenols in mg GAE/g	Total tannins in g/L	Total Flavonoids in mg QE/g
Twig extract	171.0±1.59	27.65±0.86	8.2±0.14
Bark extract	226.3±0.57	53.34±0.24	7.1±0.03
Leaf extract	191.5±0.91	11.46±0.29	31.1±0.03

Antibacterial activity

Table 3 showed that the barks and leaves extracts exhibited a potent antibacterial activity against the tested microorganisms with the MIC values ranging from 62.5 to 500 μg/mL while the twig extracts had moderate effect on the tested bacteria with the MIC values which varied from 125 to 500 μg/mL. The reference drug which is Gentamycin inhibited bacteria with the MIC which ranged from 0.03 to 0.19 μg/mL. According to Kuete (2010), the activity of plant extract is significant if MIC $\leq$ 100 μg/mL, moderate if 100 < MIC $\leq$ 625 μg/mL and low or negligible if MIC > 625 μg/mL [15].

Table 3 showed that the bark extracts were most active than the leaf and twig extracts. This could be explained by the fact that the bark had the highest tannin contents. That is in agreement with the previous work which linked antibacterial activity and tannin contents of plant extract [16]. The barks extracts had significative activity on *S. aureus* and moderate activity against *E. coli, S. typhi, S. pneumoniae, P. aeruginosa and K. pneumoniae*. The bark ethanolic extract of *B. scleroneura*w as most active against *S. aureus* (62.5 μg/mL) compared with the antibacterial activity of the bark of *B. ferruginea* against the same micro-organism (5mg/mL) [17]. Even the ethanolic extract of the leaves of *B. scleroneura* was more active than the ethanolic extract of the leaves of *B. ferruginea* against *E. coli* (50 mg/mL) and *S. aureus* (50 mg/mL) [18]. This study is in agreement with the results of the antibacterial activity of *B. cathartica* with MIC values at 250-500 μg/mL [21].

Table 3: Minimum Inhibitory Concentrations (MIC) express in (μg/mL) of the extracts

Samples	*S. aureus* ATCC 25923	*S. aureus* ATCC 43300	*S. pneumoniae* ATCC 461916	*P. aeruginosa* HM801	*K. pneumoniae* Clinical isolate	*S. typhi* clinical isolate	*E. coli* ATCC25322
MBE	62.5	62.5	125	-	-	-	500
HBE	62.5	62.5	125	500	500	500	125
MTE	250	125	-	-	-	-	500
MLE	250	62.5	250	-	-	-	250
EBE	62.5	62.5	125	500	500	500	125
HTE	250	125	250	-	-	-	-
ETE	500	125	500	-	-	-	500
ELE	250	125	250	-	-	-	500
Gentamycin	0.03	0.07	0.09	0.048	0.03	0.048	0.19

Legend: MBE:Methanolic Barkextract; HBE: Hydroethanolic Bark extract; EBE: Ethanolic Bark extract; MTE: Methanolic Twig extract; HTE: Hydroethanolic Twig extract; ETE: Ethanolic Twig extract; MLE: Methanolic Leaf extract; ELE: Ethanolic Leaf extract.

Conclusion

The current study carried out on the leaves twigs and barks of *Bridelia scleroneura* reported the phytochemical screening, chemical contents and antibacterial activity. The phytochemical screening showed the presence of alkaloids, saponins, terpenes, steroids, polyphenols, tannins, quinones and flavonoids. The highest values of polyphenol and tannin contents were observed in the barks. The leaves showed the highest flavonoid content. To the best of our knowledge, this is the first report on the total phenolic, flavonoid and tannin contents of *B. scleroneura.* The bark extract had a significant activity on *S. aureus*. Since the barks extract exhibited a significant activity on *S. aureus*, the next study will be focused on the steps of making a preformulation.

Acknowledgements

This work was financially supported by the German Academic Exchange Service (DAAD) with funds from the Federal Ministry for Economic Cooperation and Development (BMZ) through the Yaoundé-Bielefeld Graduate School of Natural Products with Antibacterial activities (YABiNaPA). We are grateful to Professor LENTA NDJAKOU Bruno, the laboratory manager of LaSuNITSO and Professor NGOUELA Silvère Augustin, the laboratory manager of LSNSO. We thank also the universities of Yaoundé I and Bielefeld for their facilities.

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