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Phytochemical Screening and *in Vitro* Evaluation of the Antibacterial Activity of Organic Extracts from the Root Bark of *Cussonia arborea* (Araliaceae)

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Abstract

Objective: To assess the usefulness of *Cussonia arborea* in the treatment of bacterial infectious diseases.

Study Design: Experimental analytical study.

Place and Duration of Study: The study was done in the Laboratory of Hydrobiology and Environment of the Faculty of Sciences, University of Yaounde1; the Bacteriology Laboratory of the Yaoundé University Teaching Hospital; the Laboratory of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Medicine and Biomedical Sciences and the Laboratory of Organic Chemistry of the Faculty of Sciences of the University of Yaoundé I. The study was done in a period of six months.

Methodology: The root bark of *Cussonia arborea* was collected in the village Yambéta (Central Region, Cameroon), dried and pulverized. Thereafter, two extractions were performed by embedding 200 g of powder in 2000 mL of 96° ethanol, and in a hydro-ethanolic mixture (30/70, *v/v*), respectively. Qualitative phytochemical screening was performed. Minimum inhibitory and bacterial toxicity were determined by macro-dilution in liquid medium on *Staphylococcus aureus, Salmonella* sp*, Shigella* sp and *Proteus mirabilis* provided by the Laboratory of Hydrobiology and Environment of the Faculty of Sciences, University of Yaounde1 and the Bacteriology Laboratory of the Yaoundé University Teaching Hospital.

Results: Phytochemical screening revealed the presence of polyphenols (flavonoids, and tannins), alkaloids, quinones, saponins and, cardiac glycosides. However, coumarins were absent in the two extracts. The minimum inhibitory concentrations of the extracts ranged from 25 to 100 mg/mL, and the minimum bactericidal concentrations from 25 to 200 mg/mL. The ethanolic extract was bactericidal against *Proteus mirabilis* and *Staphylococcus aureus,* but bacteriostatic against *Salmonella* sp and *Shigella* sp. The hydro-ethanolic extract was bacteriostatic against *Shigella* sp and bactericidal against the other strains.

Conclusion: The groups of polyphenols, alkaloids, quinones, saponins and, cardiac glycosides contained in the two extracts can justify the antibacterial activity observed against *Staphylococcus aureus, Salmonella* sp*, Shigella* sp and *Proteus mirabilis*.

Keywords: *Cussonia arborea*, Phytochemical screening, Antibacterial properties

1. Introduction

According to the World Health Organization (WHO), infectious diseases are responsible for 14.7 million deaths worldwide (Madrid et al., 2017). Diarrhoeal diseases cause the death of 751 thousand children each year in the world and are among the main causes of infant mortality and morbity with a prevalence of 13.6% in Cameroon [2]. The etiology of diarrhoea can be bacterial and the germs often responsible are *Salmonella sp, Shigella sp and Staphylococcus aureus* (Levoa et al., 2015; UMVF, 2010). The management of infectious diseases is based on pharmacotherapy that offers several families of antibiotics with different spectrums and mechanisms of action (Schwarz et al., 2017). However, the medical community is faced with a growing increase in antibiotic resistance, which considerably limits patient management (Diallo et al., 2019; Duval, 2019). Therefore, traditional medicine is usually the alternative treatment allowing the discovery of new and more effective drugs (Titanji et al., 2008). The WHO recognizes that certain plant-based medicines can replace certain so-called conventional medicines (OMS, 2013). In this process of exploring the therapeutic potential of the traditional, it was observed in Yambeta, a village in Cameroon, that traditional practitioners use the bark of the roots of *Cussonia arborea for* several therapeutic purposes, particularly in the treatment of diarrhoea. It was hypothesised that extracts of this plant may relieve patients suffering from diarrhoea of bacterial origin. To this end, we proposed to characterise the phytochemical composition of *C. arborea* roots and to evaluate its antibacterial activity in order to justify its use in traditional medicine.

2. Materials and Methods

2.1 Study Area

The study took place in the Laboratory of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Medicine and Biomedical Sciences, the Laboratory of Organic Chemistry of the Faculty of Sciences of the University of Yaoundé I, the Laboratory of Hydrobiology and Environment of the Faculty of Sciences of the University of Yaounde I and the Laboratory of Bacteriology of the Yaoundé University Hospital Center (YUHC). The study was authorized by the Institutional Research Ethics Committee of the Faculty of Medicine and Biomedical Sciences (N°208/UYI/FMSB/VDRC/DAASR/CSD).

2.2 Plant Material

The plant material consisted of *C. arborea* root bark collected in Yambeta, a locality in the Mbam and Inoubou Division in the Central Region of Cameroon. It was identified at the National Herbarium in Yaoundé under N° 11087SRF/Cam. The plant was dried in a rack for 21 days and then pulverized.

2.3 Biological Material

Bacterial strains of *Staphylococcus aureus*, *Salmonella sp*, *Shigella sp* and *Proteus mirabilis* provided by the Laboratory of Hydrobiology and Environment of the Faculty of Science of the University of Yaounde I and the Laboratory of Bacteriology of the (YUHC) were used.

2.4 Plant Extraction

Two extractions by maceration for 72 hours were performed. The first one by homogenizing 200 g of *C. arborea* root bark powder in 2000 mL of 96° ethanol. The second extraction was carried out by embedding the same mass of powder in the same volume of a hydro-ethanol

mixture (30/70, *v/v*). After filtration with the Whatmann paper n°1, the macerate obtained was dried in an oven at 40°C.

2.5 Phytochemical Screening

The phythochemical screening was perfomed according to Sofowora et al. (1993).

2.6 Antibacterial Activity

2.6.1 Preparation of Bacterial Inocula

The bacterial incula were prepared according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI) (2012).

2.6.2 Preparation of Stock Solutions of Extracts

In particular, the hydro-ethanolic and ethanolic extracts were dissolved in dimethylsulphoxide 1%. The concentration of the stock solutions was prepared at 400 mg/mL.

2.6.3 Determination of the Minimum Inhibitory and Bactericidal Concentrations of Extracts

It was carried out according to liquid macro-dilution as described by CLSI (2012).

2.7 Statistical Analysis

Data were analyzed using Word and Excel 2016 (Microsoft Office 2016, USA). Describe statistics involved the presentation of data as the percentage in tables and graph and as mean ± standard errors on the mean (SEM) for the variables analyzed.

3. Results

Table 1. Major groups of secondary metabolites present in *C. arborea* root bark extracts

Secondary metabolites	**Hydro-ethanolic extracts**	**Ethanolic extracts**
Alkaloids	+	+
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Saponosid	+	-
Steroids et triterpens	+	+
Anthocyanins	+	+
Coumarins	-	-
Quinones	+	+
Cardiac glycosides	+	+

+ Present; - Absent

The hydro-ethanolic extract showed the presence of all tested secondary metabolite groups except coumarins. The ethanolic extract showed the absence of saponosides and coumarins.

3.2 Antibacterial Activity

Table 2. Inhibition parameters of *C. arborea* root bark extracts.

Microorganisms	Ethanolic extract			Hydro-ethanolic extract		
	CMI	CMB	CMB/CMI	CMI	CMB	CMB/CMI
Proteus mirabilis	100	200	2	100	200	2
Staphylococcus aureus	25	25	1	100	100	1
Shigella sp	25	100	4	50	200	4
Salmonella sp	50	200	4	100	200	2

Ethanolic and hydro-ethanolic extracts of *C. arborea* root bark were active on the different bacterial strains tested. The minimum inhibitory concentrations were between 25 and 100 mg/mL, and the minimum bactericidal concentrations between 25 and 200 mg/mL. The ethanolic extract was found to be bactericidal on *Proteus mirabilis* and *Staphylococcus aureus*, but on *Salmonella* sp and *Shigella* sp it was bacteriostatic. The hydro-ethanolic extract was bacteriostatic against *Shigella* sp and bactericidal against the other strains.

4. Discussion and Conclusion

The present study focused on the phytochemical characterization and evaluation of antimicrobial activity of the hydro-ethanolic and ethanolic extracts of the root bark of *C. arborea*. The phytochemical analysis performed on the extracts showed that the hydro-ethanolic extract and the ethanolic extract of *C. arborea* root bark have similar compounds. However, saponins were higher in the ethanolic extract than in the hydro-ethanolic extract, results in agreement with findings by Tabouguia et al. (2014) who did not observe the presence of saponins in the hydro-ethanolic extract. This difference might be justified by the fact that the latter used methanol as extraction solvent. Besides, in comparison to the results obtained by Tabouguia et al. (2014), alkaloids were revealed in both extracts. Tabouguia et al. had indeed used the Meyer test to measure alkaloid levels while in our study, the Hager and Draggendorf tests were used.

The second objective of the present work was to evaluate the antibacterial activity of ethanolic and hydro-ethanolic extracts of *C. arborea* root bark. These extracts were all active against *S. aureus, Salmonella sp, Shigella spp and P. mirabilis.* The phytochemical composition of the extracts may justify this activity. Many studies have shown that tannins, steroids, flavonoids, quinones, saponins, and terpenoids possess antimicrobial properties (Salihu, 2008; Cowan, 1999). Indeed, these secondary metabolites act through different mechanisms of action in infectious diseases. For instance, Tannins act by depriving iron (Scalbert, 1991), alkaloids intercalate into DNA and inhibit its synthesis. Compean et al. (2014) had shown that steroids, saponins and tannins present in plant extracts may justify their bactericidal potential, findings that are consistent with the results obtained in our study.

Strength of the study

From a pharmacological point of view, this study justifies the traditional use of *Cussonia arborea* in the treatment of diarrhoea.

Weakness of the study

This study did not identify the group of secondary metabolites responsible for the antibacterial activity and did not provide any toxicological data of the plant extracts.

Disclosure statement: The authors declare no conflict of interest.

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