In Vitro Study of the Antibacterial Activity of Extracts of *Boscia senegalensis* (Pers) Lam. Ex Poir. (Capparaceae) collected in Mali

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ABSTRACT: For thousands of years, humanity has used plants from its environment to treat diseases. These plants contain chemical compounds with biological activities. *Boscia senegalensis* is among the medicinal plants used by rural populations in Mali. The objective of this study was to determine the secondary metabolite composition and evaluate the antibacterial activity of leaf and stem extracts. The plant material consisted of leaves and stems collected in April 2023 in Niono, Segou region, Mali. The biological material included bacterial strains: *Escherichia coli, Proteus vulgaris, Salmonella typhi, Staphylococcus saprophyticus, Enterococcus hormaechei, and Candida albicans*. The extracts were prepared by reflux heating and Soxhlet extraction. Colorimetric reactions were used to identify the chemical constituents of the powders and extracts. Antibacterial activity was assessed by measuring (in mm) the diameter of the inhibition zones at different concentrations. Alkaloids, saponosides, tannins, and terpenoids were identified in the powders and extracts. The aqueous extract of stems and leaves showed significant antibacterial activity against S. saprophyticus at 100 mg/mL (inhibition zones of 15.5 mm and 10 mm, respectively) and 9 mm at 75 mg/mL. Further research is needed to identify the active compounds responsible for this activity and explore resistance mechanisms in the other microbial strains studied.

Keywords: *Antibacterial activity, Boscia senegalensis, phytochemistry*

I. INTRODUCTION

For thousands of years, humans have relied on a wide variety of plants from their natural environment to treat and cure diseases [1]. These plants constitute an immense reservoir of compounds with a high diversity of chemical structures and a broad spectrum of biological activities [2]. However, evaluating these bioactivities remains an area of great scientific interest and has prompted numerous studies. Many different natural substances have been identified, and a large number are traditionally used for the prophylaxis and treatment of diseases [3].

Currently, the World Health Organization (WHO) estimates that approximately 81% of the global population relies on traditional plant-based remedies as primary healthcare [4]. Herbal medicines remain widely used and hold considerable importance in the global pharmaceutical market. More than 120 compounds derived from plants are currently used in modern medicine, and around 75% of them are used in ways consistent with traditional practices [5]. Among the 25 most sold pharmaceutical drugs

worldwide, 12 are derived from natural products [6]. This highlights the potential of natural product research in drug discovery, as it often requires screening far fewer molecules compared to combinatorial chemistry, where over 10,000 compounds must be synthesized and tested to develop a single drug.

Of the approximately 250,000 to 300,000 plant species identified globally, only 5 to 15% have been studied for bioactive molecules [7], suggesting that the plant kingdom represents a vast source of potential new medicinal compounds.

The antimicrobial properties of medicinal plants have been recognized since antiquity. Despite its long history, the study of plant chemistry remains a highly relevant research area due to the extensive variety of bioactive molecules produced by plants [8]. These substances find application across industries in food, cosmetics, pharmaceuticals and beyond. Notable classes of such compounds include coumarins, alkaloids, phenolic acids, tannins, terpenes and flavonoids [9].

In this context, we focused our attention on *Boscia senegalensis*, a shrub from the Capparaceae family. Native to the Sahel region of Africa, this evergreen dicotyledonous plant grows in favorable conditions across various environments [10]. In Mali, *B. senegalensis* is commonly used as both food and medicine by rural populations [11]. The greenish flesh of unripe fruits is cooked and consumed like peas or ground into cakes or couscous. The fruits are widely appreciated and consumed, particularly in the Sahel. The leaves are also used as livestock fodder, especially for cattle [12]. Various parts of *B. senegalensis* including fruits, leaves, roots, and stems are traditionally used to treat ailments such as cough, malaria, swelling, cancer, rheumatism, ulcers, jaundice and sexually transmitted infections [13]. Given its multiple medicinal and nutritional uses, it is worthwhile to investigate the biological activities of some of its major phytochemical groups.

The aim of the present study was to characterize the phytochemical composition and evaluate the antibacterial activity of extracts from the leaves and stems of *B. senegalensis*.

II. MATERIAL AND METHODS

1 Plant material

The leaves and stems of *Boscia senegalensis* were collected on April 17, 2023, in Niono (Ségou Region, Mali). The plant was identified by a botany specialist at the Institute of Research in Traditional Medicine and Traditional Pharmacopoeia (INRMPT), formerly the Department of Traditional Medicine (DMT), under the herbarium number 0735/DMT. The samples were air-dried for two weeks and then pulverized into fine powder for extraction.

2 Biological material

The bacterial strains used were pathogenic strains from ATCC: three Gram-negative strains (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 35659, *Salmonella typhi*), two Gram-positive strains (*Staphylococcus saprophyticus* ATCC BAA-750 and *Enterococcus hormaechei* ATCC 700323), and one fungal strain (*Candida albicans*). All strains were stored at 5°C in sterilized tubes filled with 10 mL of slanted nutrient agar.

3 Mueller-Hinton (MH) Agar Medium

Mueller-Hinton agar was used to assess the bacterial susceptibility to natural extracts. This solid medium also serves as an excellent base for blood agar preparation. The composition is as follows:

- Beef extract: 300 mL. L^{-1}
- Casein peptone: 17.5 g. L^{-1}
- Corn starch: 1.5 g. L^{-1}
- Agar: 17 g. L^{-1} [14].

4 Extract preparation

4.1 Aqueous decoction: Prepared by refluxing 10 g of powdered plant material in 100 mL of distilled water for 15 minutes.

4.2 Methanolic extract: Prepared using Soxhlet extraction with 40 g of powdered material in 400 mL of methanol.

5 Phytochemical screening of secondary metabolites

Colorimetric tube reactions with specific reagents were used to identify the main phytochemical groups in the powders and aqueous extracts of leaves and stems.

(Due to length, this subsection and the rest will be continued in the next message).

5.1 Plant powder

Detection of Total Polyphenols

Two grams of plant powder were added to 50 mL of boiling distilled water. After cooling, the solution was filtered. Two milliliters of the filtrate were transferred to a test tube, and 1–2 drops of 2% ferric chloride ($FeCl_3$) were added. The appearance of an intense blackish or black-green precipitate confirmed the presence of polyphenols [15].

Detection of Flavonoids (Shibata or Cyanidin Test)

Ten grams of powder were boiled in 50 mL of distilled water for 30 minutes. The hot solution was filtered through cotton and allowed to rest. Two milliliters of the filtrate were placed in a test tube, followed by 2 mL of hydrochloric alcohol and a few magnesium turnings. The appearance of an orange-pink or pink-purple color indicated the presence of flavonoids [15].

Detection of Tannins

Five grams of powder were added to 50 mL of boiling water in a 250 mL flask and left to rest for 30 minutes. After cooling and filtering, 5 mL of the infusion were mixed with 4 mL of Stiasny's reagent (formalin-HCl) and heated in a water bath until boiling for 30 minutes. A precipitate indicated condensed tannins. After filtration, the filtrate was saturated with sodium acetate, followed by a few drops of 2% $FeCl_3$. The appearance of a blue-black color confirmed hydrolyzable tannins.

To detect catechic tannins (non-hydrolyzable), 1 mL of concentrated hydrochloric acid was added to 5 mL of the same infusion and the mixture was boiled for 15 minutes. The presence of a red precipitate insoluble in amyl alcohol confirmed catechic tannins [15].

Detection of Alkaloids

One gram of powder was macerated in 15 mL of 10% sulfuric acid for 30 minutes at room temperature and filtered. One milliliter of the filtrate was distributed into three test tubes:

- Tube 1: 2–3 drops of Dragendorff's reagent;
- Tube 2: 2–3 drops of Bouchardat's reagent;
- Tube 3: 2–3 drops of Valser-Mayer's reagent.

A red-orange precipitate in tube 1, a brown precipitate in tube 2 and a pale yellowish-white precipitate in tube 3 confirmed the presence of alkaloids [15].

Detection of Cardiotonic Glycosides

One gram of powder was mixed with 5 mL of a chloroform/ethanol (4:1 v/v) solution. After 30 minutes of maceration with occasional shaking, the filtrate was divided into three test tubes:

- Tube 1: 0.5 mL of Baljet reagent;
- Tube 2: 0.5 mL of Kedde reagent;
- Tube 3: 0.5 mL of Raymond-Marthoud reagent.

Two drops of diluted caustic soda in 90% ethanol were added to each, and pH was checked. An orange color in tube 1, a stable purple-red in tube 2 and a transient violet color in tube 3 confirmed the presence of cardiotonic glycosides [15].

Detection of Sterols and Triterpenes

One gram of powder was macerated in 20 mL of diethyl ether for 24 hours, then filtered. Ten milliliters of the filtrate were evaporated to dryness at room temperature, and the residue was dissolved in 1 mL of acetic anhydride and 1 mL of chloroform. Two test tubes were used: one as a blank, and in the other, 1–2 mL of concentrated sulfuric acid were added. A reddish-brown or violet ring at the interface, with a green supernatant, indicated sterols and triterpenes [16].

Detection of Carotenoids

Five milliliters of the ether macerate were evaporated to dryness in a water bath. Then, 2–3 drops of saturated antimony trichloride solution (prepared by dissolving 2–3 pieces of antimony in 5 mL of chloroform) were added. A blue coloration followed by opalescent turbidity confirmed the presence of carotenoids [16].

Detection of Reducing Compounds

Five milliliters of a 10% aqueous decoction were evaporated to dryness. A few drops of Fehling's reagent were added. The formation of a brick-red precipitate indicated the presence of reducing sugars [15].

Detection of Saponosides

A 1% aqueous decoction was filtered and diluted to 100 mL, then allowed to cool. Ten test tubes numbered 1 to 10 were filled with increasing volumes (1–10 mL) of decoction and topped up to 10 mL with distilled water (except tube 10). Each tube was shaken longitudinally for 15 seconds (2 shakes per second), and after 15 minutes, foam height was measured. The tube where foam reached 1 cm served as a reference.

Foam Index = 100 / X; X = foam height in cm [15].

Detection of Anthracene Derivatives

A hydro-acid solution was prepared by mixing 250 mg of powder, 20 mL of distilled water, and 1 mL of concentrated HCl. After boiling in a water bath for 15 minutes, the mixture was cooled and filtered. The filtrate was transferred to a separating funnel, and 10 mL of chloroform were added. The chloroform phase was evaporated to dryness and the residue was dissolved in 2 mL of dilute ammonia (1:2). A yellow-to-red color change after mild heating confirmed the presence of anthracene derivatives [16].

5.2 Extracts

Detection of Flavonoids

One milliliter of each extract was treated with a few drops of concentrated HCl. A few magnesium turnings were added. The appearance of a pinkish-red color confirmed the presence of flavonoids [16].

Detection of Tannins

To 1 mL of each extract, 2–3 drops of 1% $FeCl_3$ solution were added. After a few minutes of incubation, the appearance of a dark blue or green color indicated the presence of tannins [16].

Detection of Alkaloids

To 2 mL of each extract, 5 mL of 1% HCl were added. The mixture was heated in a water bath. Each sample was divided into two portions: Mayer's reagent was added to one, Wagner's reagent to the other. The formation of white and brown precipitates respectively confirmed the presence of alkaloids [15].

Detection of Sterols and Triterpenes

To 1 mL of each extract, 1 mL of acetic anhydride and a few drops of concentrated H_2SO_4 were added. The appearance of a violet to green color, or a reddish-brown ring at the interface, confirmed the presence of sterols and triterpenes [16].

Detection of Terpenoids

One milliliter of each extract was treated with 0.4 mL of concentrated H_2SO_4. The appearance of two layers and a brown coloration at the interface indicated the presence of terpenoids [16].

Detection of Anthraquinones

To 1 mL of each extract, 0.5 mL of 10% NH_4OH was added and the mixture was shaken. The appearance of a violet color indicated a positive test [16].

Detection of Reducing Sugars

Five milliliters of each extract were mixed with 1 mL of Fehling's solution (A+B) and heated in a water bath for 5 minutes. The formation of a brick-red precipitate confirmed the presence of reducing sugars [15].

Detection of Saponosides

One milliliter of each extract was mixed with 2 mL of warm distilled water. The mixture was shaken for 15 seconds and allowed to rest for 15 minutes. The formation of a persistent foam layer of more than 1 cm indicated the presence of saponosides [15].

6 Preparation of the inoculum

Antibacterial activity was tested using young bacterial cultures in the exponential growth phase. To reactivate the strains, they were subcultured by streaking on pre-poured nutrient agar in Petri dishes, then incubated at 37°C for 18–24 hours.

To prepare the inoculum, 3–5 similar, well-isolated colonies were collected using a sterile platinum loop and transferred into sterile physiological saline. The bacterial suspension was homogenized using a vortex mixer, and the turbidity was adjusted to 0.5 McFarland, corresponding to an optical density of 0.10 at 620 nm, equivalent to 10^8 CFU/mL [17].

7 Preparation of extract dilutions

For the preparation of the various concentrations of lyophilized extracts, 100, 75 and 50 mg of each extract were introduced into sterile Eppendorf tubes containing 1 mL of DMSO. The tubes were

vigorously shaken using a vortex until the solutions were homogeneous.

For each of the two plant parts (stems and leaves), stock solutions were prepared (*Boscia* Stem + DMSO and *Boscia* Leaf + DMSO). These stock solutions were then progressively diluted in DMSO to prepare a range of working solutions with final concentrations of 100, 75 and 50 mg/mL. These solutions were used to assess antibacterial and antifungal activities.

The disc diffusion method was used to evaluate the antibacterial activities of the extracts. In sterile Petri dishes, 20 mL of Mueller-Hinton agar were poured and allowed to solidify for 20 minutes.

Once solidified, 1 mL of bacterial suspension at 10^8 CFU/mL was spread evenly over the surface of each agar plate [18].

Sterile Whatman No. 1 paper discs (6 mm in diameter) were impregnated with 5 µL of each extract at concentrations of 50, 75 and 100 mg/mL and placed on the surface of the inoculated agar [19, 20].

The plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the diameter (in mm) of the inhibition zones formed around each disc using a ruler. A 10% aqueous solution of DMSO was used as the negative control. Each test was performed in triplicate under the same conditions, and mean values were recorded [14].

III. RESULTS

1 Phytochemical composition

The table I reports the outcomes of phytochemical tests, which revealed the presence of several bioactive metabolites in *Boscia senegalensis*.

Table I: Phytochemical Composition of *Boscia senegalensis*

Phytochemical Constituents	Powder		Aqueous Extract	
	Leaves	Stems	Leaves	Stems
Total polyphenols	+	+	+	+
Flavonoids	-	+	-	-
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Saponosides	+	+	+	+
Sterols and triterpenes	+	-	+	-
Carotenoids	-	+	-	-
Anthracene derivatives	-	-	-	-
Reducing compounds	-	-	-	-
Terpenoids	+	+	+	+

(+ indicates positive reaction; – indicates negative reaction)

Phytochemical screening confirmed the presence of flavonoids (in stem powder), tannins, alkaloids, saponosides and terpenoids.

2 Antibacterial activity

The antibacterial activity results are shown in Tables II and III.

Table II: Antibacterial Test Results for Aqueous Stem Extract

Diameter of inhibition zones (mm), minimal inhibitory concentrations and bacterial strain sensitivity

Diameter of inhibition zones (mm), minimal inhibitory concentrations and bacterial strain sensitivity												
		E. coli		*S. saprophyticus*		*E. hormaechei*		*P. vulgaris*		*S. typhi*		*C. albican*	
	mg/mL	D	S	D	S	D	S	D	S	D	S	D	S
Aqueous	100	7	-	**15,5**	++	7	-	7	-	7	-	7	-
Extract	75	7	-	**9**	+	7	-	6	-	6	-	6	-
+DMSO	50	6	-	6	-	6	-	6	-	6	-	6	-
	mg/mL	D	S	D	S	D	S	D	S	D	S	D	S

Aqueous	100	6	-	**9**	-	6	-	6	-	6	-	6	-
Extract	75	6	-	6	-	6	-	6	-	6	-	6	-
+Water	50	6	-	6	-	6	-	6	-	6		6	-
Witness													
DMSO	--------	D	S	D	S	D	S	D	S	D	S	D	S
	---------	6	-	6	-	6	-	6	-	6	-	6	-

Table III: Antimicrobial Test Results for Aqueous and Methanolic Leaves Extract

Diameter of inhibition zones (mm), minimal inhibitory concentrations and bacterial strain sensitivity													
		E. coli		*S. saprophyticus*		*E. hormaechei*		*P. vulgaris*		*S. typhi*		*C. albican*	
	mg/mL	D	S	D	S	D	S	D	S	D	S	D	S
Aqueous	100	7	-	**10**	+	7	-	7	-	7	-	7	-
Extract	75	7	-	**9**	+	7	-	6	-	6	-	6	-
+DMSO	50	6	-	6	-	6	-	6	-	6	-	6	-
	mg/mL	D	S	D	S	D	S	D	S	D	S	D	S
Methanolic	100	6	-	**9**	-	6	-	6	-	6	-	6	-
Extract	75	6	-	6	-	6	-	6	-	6	-	6	-
+Water	50	6	-	6	-	6	-	6	-	6		6	-
Witness													
DMSO	--------	D	S	D	S	D	S	D	S	D	S	D	S
	---------	6	-	6	-	6	-	6	-	6	-	6	-

DMSO: Negative control; D: Diameter of the inhibition zone; S: Sensitivity; –: Resistant; +: Sensitive; ++: Very sensitive; +++: Extremely sensitive

(–): Resistant (Ø < 8 mm); (+): Sensitive (9 mm < Ø < 14 mm); (++): Very sensitive (15 mm < Ø < 19 mm); (+++): Extremely sensitive (Ø > 20 mm

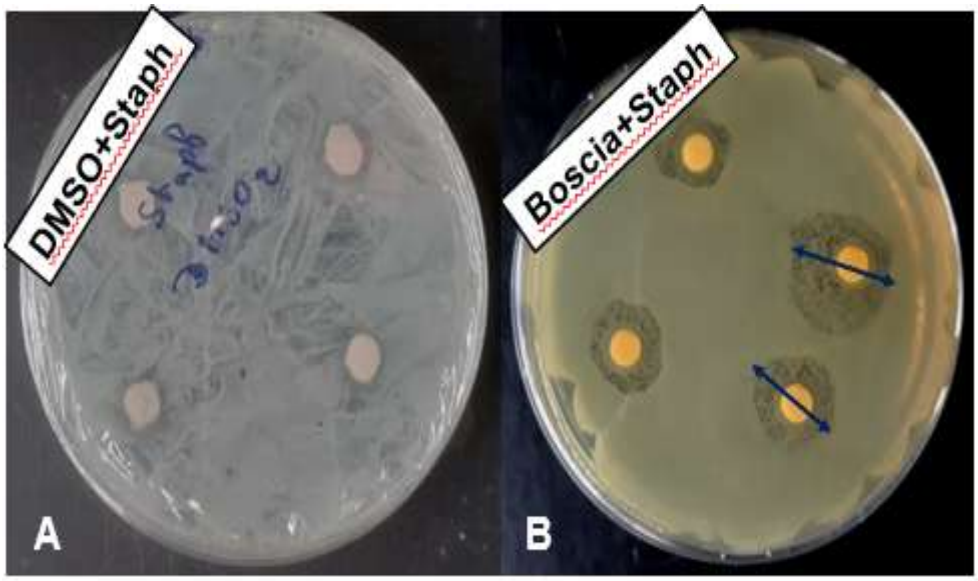

Fig 1: Antimicrobial activity of the aqueous stem extract (Cissé. S, 2023) **A**: Control (discs impregnated with DMSO); **B**: Test (discs impregnated with the aqueous stem extract of *B.*

senegalensis).

The antibacterial activity results showed that the aqueous extracts from the stems and leaves of *B. senegalensis* exhibited significant activity against the Gram-positive bacterial strain *S. saprophyticus* at 100 mg/mL (inhibition zones of 15.5 mm and 10 mm, respectively) and 9 mm at 75 mg/mL in both cases.

IV. DISCUSSION

Phytochemical screening of the powders and aqueous extracts from leaves and stems confirmed the presence of alkaloids, saponosides, tannins and terpenoids. Flavonoids and carotenoids were detected only in the stem powder. These findings align with the work of Chaibou [21], who reported tannins and alkaloids in *B. senegalensis*, establishing these as major chemical constituents. However, Osuala *at al.,*[22] did not detect flavonoids in their extracts, likely due to differences in extraction methods, environmental growth conditions, or geographic origin which can all influence phytochemical profiles.

Additional phytochemical analyses by Awa *at al.,*[23] on pulp and seeds identified saponosides, alkaloids, sterols, triterpenes and phenolic compounds including flavonoids. Adam *at al*.,[24] similarly detected saponosides and alkaloids in seed extracts from Chad, although no flavonoids, triterpenes, or sterols were found. In root extracts, Aliyu (2008) identified alkaloids, flavonoids, saponins, tannins and cardiotonic glycosides. Diatta [3] reported alkaloids, saponosides, flavonoids and tannins in both aqueous and alcoholic extracts.

The alkaloids in *B. senegalensis* may contribute to its known antimicrobial and antiparasitic activities [25]. Saponosides, being amphiphilic glycosides, have detergent-like properties and are linked to anti-inflammatory, antimicrobial, hemolytic activities, enhanced digestion and immunostimulation [2, 21, 26]. Tannins phenolic compounds known for antioxidant, astringent, antimicrobial and antitumor effects likely impart antiseptic properties beneficial in wound healing [16]. Terpenoids may explain the plant's use as a stimulant or tonic in traditional medicine.

Antibacterial testing revealed that *S. saprophyticus* showed notable sensitivity (15.5 mm inhibition zone) to the aqueous stem extract in DMSO at 100 mg/mL a finding consistent with Bah *at al*.,[27]. This suggests the presence of potent bioactive compounds in the stem, with a dose-response relationship evident by a reduced zone (9 mm) at 75 mg/mL. In contrast, the leaf extract showed weaker activity, which agrees with Vougat *at al.,*[10]. who also found no antibacterial effect of hexanoic or methanolic leaf extracts against *Staphylococcus* species. These results highlight significant chemical differences between stems and leaves, particularly higher concentrations of active secondary metabolites in stems.

The observed resistance of other tested microbial species, even at high concentrations (up to 100 mg/mL), might stem from their cell wall characteristics limiting extract penetration or the inability of the compounds to disrupt vital microbial processes.

Importantly, the pronounced activity against *S. saprophyticus* using aqueous stem extracts without organic solvents suggests potential formulations for contexts where solvents like DMSO are undesirable. This indicates that *B. senegalensis* extracts may serve as a natural alternative to conventional antibiotics for specific infections.

Cameroonian studies reported significant inhibition of *S. aureus* by hexane extracts of *B. senegalensis*, with inhibition zones of 16 mm at 20 mg/mL, 14.75 mm at 10 mg/mL and 13.25 mm at 5 mg/mL [10]. The similarity between these results and our 15.5 mm zone at 100 mg/mL supports the hypothesis that hexane extracts might contain molecules functioning similarly to penicillin.

Given the known antibacterial properties of compounds such as tannins, saponins and alkaloids, and considering solvent effects, the activity observed in this study may indeed be attributed to these components [28, 29]. Root extract analyses [24] also reported antimicrobial constituents reinforcing the therapeutic potential of *B. senegalensis*. However, antibacterial efficacy is clearly influenced by plant part, extraction solvent, and microbial species tested. While results are promising, further investigations including clinical trials are needed to confirm safety and efficacy.

V. CONCLUSION

Boscia senegalensis exhibits promising antibacterial potential due to its bioactive compounds alkaloids, flavonoids, saponins and tannins. Specifically, the aqueous stem extract showed significant activity against *Staphylococcus saprophyticus*. Despite these encouraging findings, further research is essential to elucidate the mechanisms of action, broaden the antimicrobial spectrum and assess safety through clinical evaluations. Ultimately, this plant could offer a natural alternative to conventional antibiotics, especially in regions where it has traditional medicinal use.

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