



Preliminary Biological Study of Two Medicinal Plants used in the Mouhoun Region (Burkina Faso): *Boscia angustifolia* A. Rich (Caparaceae) and *Gardenia erubescens* Stapf & Hutch (Rubiaceae)

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Authors' contributions

The design of the research idea was carried out with the support of author's AH and JYPN. The phytochemistry and the antioxidant tests were coordinated by authors JYPN and DP. For the benchwork of authors JYPN, DP and MN participated. Author JYPN has contributed to the writing and editing of this work.

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ABSTRACT

Aims: *Boscia agustifolia* and *Gardenia erubescens* are two medicinal plants widely used in the Mouhoun region. The strong use of these two plants in traditional medicine would be linked to their therapeutic virtues.

Study Design: The purpose of this work was to carry out a preliminary biological study on two plants widely used by the population of the Mouhoun region (Burkina Faso) against certain diseases.

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Place and Duration of Study: The harvest of plant material was made in the Mouhoun region in March 2019. The phytochemistry and the antioxidant tests were carried out at LABIOCA in June 2019. The microorganism tests were carried out at CRSBAN.

Methodology: The extracts were obtained by ethanolic maceration. The FRAP method and DPPH were used to evaluate the antioxidant properties of the extracts. The antimicrobial activity of the extracts was determined using five microbial strains.

Results: The ethanolic extract of *Gardenia erubescens* bark had antioxidant activity through the iron ion reduction capacity (6.71 ± 1.16 mMol EAA/100 g extract). *Boscia angustifolia* showed inhibitory activity on the five microbial strains.

Conclusion: The biological activities obtained with these extracts could be justified by the presence of active phytochemicals such as flavonoids. These biological properties to constitute a reason based on the strong use of these two plants in traditional medicine.

Keywords: Phytochemistry; antioxidant; antimicrobial activity; medicinal plants; Burkina Faso.

1. INTRODUCTION

For several decades, plants have always been an important source for men. While some are used as foods, as medicines, others are used for construction or as firewood [1]. The purpose of this work was to carry out a preliminary biological study on two plants widely used by the population of the Mouhoun region (Burkina Faso) against certain diseases.

Boscia angustifolia is an evergreen tree up to 10 m tall. The leaves of this tree are arranged alternately or fasciculated by 2-4, elliptical oblanceolate or oblong. These leaves are leathery, rounded to cuneiform. *Boscia angustifolia* (*B. angustifolia*.) has ovate sepals (2-5 mm long), with 5-9 stamens, clustered in dense subterminal racemes. The different parts of this plant are used to relieve the population of certain diseases. According to Malgra [2], *Boscia angustifolia* could be used against headaches, neuralgia, kidney problems, schistosomiasis, gonorrhoea, uterine tumor, cholagogues, rheumatism, sexual disorders. According to other authors, this plant is used specifically against hepatobiliary diseases [3]. The leaves are used for several therapeutic purposes such as anti-tumor [4], cytostatic, vulnerary, anti-inflammatory, analgesic, diuretic, cicatrisant, antiseptic, osteoarthritis, rheumatic pain, ulcers, swelling, ophthalmia, migraines, headache, guinea worm, gonarthrosis [5].

Gardenia erubescens is a low-growing tree with many ramifications that look like a bush that could reach 1 to 3 m in height [6]. The leaves are grouped in a tuft at the end of short branches. The flowers are white turning to creamy yellow when ripe. The fruits consist of very tight fibers, consisting essentially of wood with at the top

points corresponding to the lobes of the dried calyx [7]. This plant is widely used as food, but also for these therapeutic virtues. According to some authors [5], *Gardenia erubescens* (*G. erubescens*) trunk bark is used against weight-loss delays, diseases of the stomach and intestines, poisoning, gastro-intestinal gastric and gastro-enteritis [8].

A survey carried out in the Mouhoun region revealed that both plants were used by herbalists and traditional healers in the management of liver diseases [9]. This study has allowed through phytochemistry to highlight certain chemical groups, and through some biological activities such as antioxidant and antimicrobial properties highlight some potentialities of extracts from *Gardenia erubescens* and *Boscia angustifolia*.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *Boscia angustifolia* and the bark of *Gardenia erubescens* were harvested in the Mouhoun region. Herbaria were made and deposited at the Biodiversity Laboratory at University Joseph KI-ZERBO under the following codes: *Boscia angustifolia* (16738) and *Gardenia erubescens* (16731). The harvested samples were dried in the shade and pulverized. The powder was used for extraction.

2.2 Extraction by Ethanol Maceration

The powder was mixed with absolute ethanol in the ratio 1:10. The mixture in the jar was stirred for 24 hours. The filtrate was recovered after 24 hours and then concentrated in a rotavapor. The resulting concentrate was subsequently dried in a ventilated oven at 40°C.



Photo 1. *Boscia angustifolia* (16738)



Photo 2. *Gardenia erubescens* (16731)

2.3 Determination of Total Phenolics

The total phenolics assay was performed based on the method of Singleton et al. [10] with some modifications. This method is based on the Folin Ciocalteu colorimetric principle. Thus the Folin Ciocalteu reagent (0.2 mol/L) was mixed with the extract. After addition of the sodium carbonate and incubation, the reaction mixture is read at 760 nm using a UV-visible spectrophotometer. Gallic acid was used as a reference through a standard curve ($y = 201x - 21.22$, $r^2 = 0.99$). The results were expressed in milligram gallic acid

equivalent / gram of extract (mg GAE/g of extract).

2.4 Determination of Flavonoids

The total flavonoid assay was done using the method of Compaoré et al. [11]. The reaction mixture consisted of $AlCl_3$ and the extract or reference compound in the proportions v / v (1: 1). After an incubation period, the reading was made at 415 nm using a standard curve ($y = 39.8x - 3.5$, $r^2 = 0.99$). The contents were expressed in milligram Quercetin Equivalent / gram extract (mg QE / g extract).

2.5 Anti-Radical Activity DPPH*

The determination of the anti-radical capacity of the extracts was made with reference to the method of Ali et al. [12]. This method is based on the reduction of the absorbance of the DPPH radical at 517 nm. The tests were repeated four times and the results expressed on mean \pm standard deviation. Quercetin was used as the reference compound.

2.6 FRAP Reducing Power

The ability of the extracts to reduce ferric ion to ferrous ion was evaluated according to the method described by Hinneburg et al. [13]. This method consists in mixing 0.5 mL of extract, 1.25 mL of phosphate buffer (0.2 M) and 1.25 mL of hexacyanoferrate potassium dissolved in distilled water (1%). After heating in a water bath at 50°C for 30 minutes and cooling, trichloroacetic acid was added. To 125 μ L aliquots to which distilled water and FeCl₃ were added. The reading was made 700 nm using a standard curve ($y = 0.0211x + 0.008$, $r^2 = 0.998$). The experiment was repeated four times independently and the results expressed in mMol equivalent of ascorbic acid per gram of extract (mMol EAA / g extract).

2.7 Antibiomicrobial Extracts Test

2.7.1 Microbial strains

Five bacterial strains including: 3 Gram-negative strains (*Escherichia coli* ATCC8739, *Salmonella typhi*, *Shigella dysenteriae* ATCC9027) and 2 Gram-positive strains (*Staphylococcus aureus* ATCC25923, *Bacillus cereus*).

2.7.2 Preparation of the inoculum

The method described by Lennette et al. [14], reported by Mihin et al. (15) was used. A suspension of each bacterial strain was prepared in 10 mL of Mueller-Hinton Broth for 18-24 hours at 37°C. Using the sterile diluent (physiological saline), the concentration was adjusted in each tube to about 1.0 10⁸ CFU / mL, comparable to that of the McFarland 0.5 standard.

2.7.3 Evaluation of the bacterial growth inhibition effect by the well methods

The diameter of the inhibition zone of the extracts was determined by the well method [15]. A volume of 10 μ L of extract (20 μ g / mL)

solubilized in 10% DMSO was placed in the wells previously made using a Pastor pipette on a MH agar inoculated by flooding with the bacterial suspension equivalent to Mac Farlan. All petri dishes were incubated for 24 hours. All tests were repeated in triplicate.

The results were read by measuring the diameters of the zones of inhibition corresponding to the light zone around the wells [16]. The sensitivity of the strains was classified according to the diameters of inhibition by Negreiros et al. [17]. Indeed, the microbial strain is insensitive to a diameter of less than 8 mm, sensitive for a diameter of between 9 mm to 14 mm, very sensitive for a diameter of between 15 mm to 19 mm and extremely sensitive for a diameter of inhibition greater than 20 mm.

2.8 Statistical Evaluation

In the tables, the data were expressed in Mean \pm SD. The graphs were drawn, and the statistical analysis was carried out, using GraphPad Prism software version 5.0 for MacOSX (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1 Phytochemistry

3.1.1 Determination of total phenolics

Fig. 1 shows in milligram equivalent gallic acid the content of total phenol extracts. The ethanolic extract of *Gardenia erubescens* trunk bark had the highest grade (1.06 \pm 0.00) compared with the ethanolic extract of *Boscia angustifolia* leaves. The statistical analysis did not show a statistical difference in the contents of the two extracts.

3.1.2 Determination of total flavonoids

The ethanolic extract of *Boscia angustifolia* had a higher total flavonoid content than the ethanolic extract of *Gardenia erubescens* trunk bark. *Boscia angustifolia* had a content of 0.21 \pm 0.001 mg QE / g extract (Fig. 2).

3.2 Biological Activities

3.2.1 Antioxidant activities

3.2.1.1 Anti-radical activity DPPH

The ethanolic extract of *Gardenia erubescens* bark had a good PFLP scavenging capacity

compared to the ethanolic extract of *Boscia angustifolia* leaves ($6.71 \pm 1.16\%$). In contrast, quercetin as the reference compound gave a percentage inhibition of 77.26 ± 1.04 (Fig. 3).

3.2.1.2 FRAP reducing power

The results on the iron ion reduction capacity of the extracts had shown that the ethanolic extract of *Boscia angustifolia* leaves was the most effective (23.82 ± 0.77 mMol EAA / 100 g extract) (Fig. 4).

3.2.2 Antimicrobial activities

Table 1 shows the inhibition diameters of the extracts according to the microbial strains. The inhibition diameters vary between 6 mm and 14 mm for the extracts. According to the recommendations of Negreiros et al. [17], the tested strains are sensitive to the ethanolic extract of *Boscia angustifolia* leaves, this is what emerges from the photo 3 where it is highlighted the action of this extract on the stump *Staphylococcus aureus* and *Salmonella thyphi*. In contrast to this extract, the ethanolic extract of

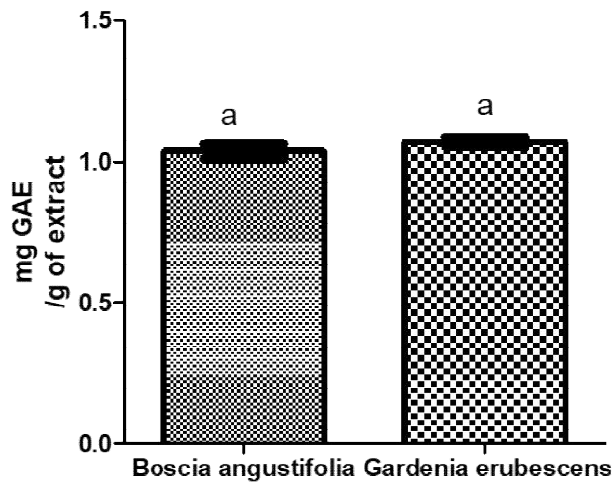


Fig. 1. Results of the determination of total phenolics
The values are represented on average \pm Standard deviation ($n = 4$)

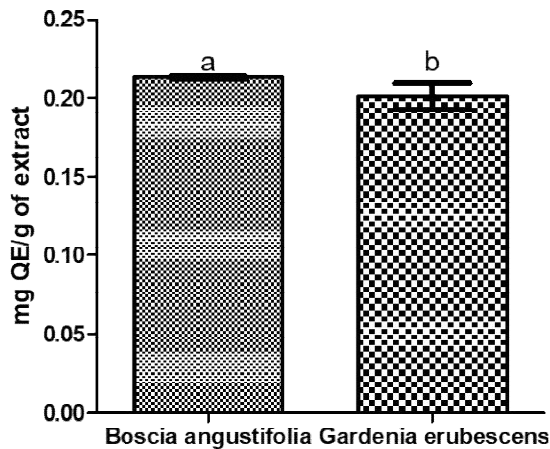


Fig. 2. Results of the determination of total flavonoids
The values are represented on average \pm Standard deviation ($n = 4$). The letters (a-b) are significantly different at $P < 0.05$

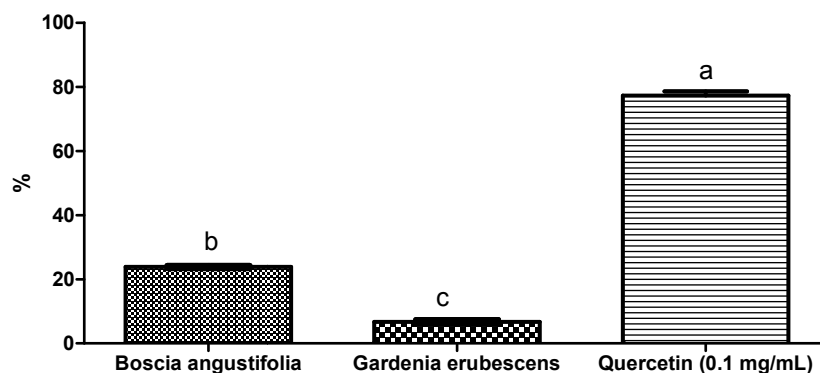


Fig. 3. Results of the determination of anti-radical activity DPPH*

The values are represented on average \pm Standard deviation ($n = 4$). The letters (a-c) are significantly different at $P < 0.05$

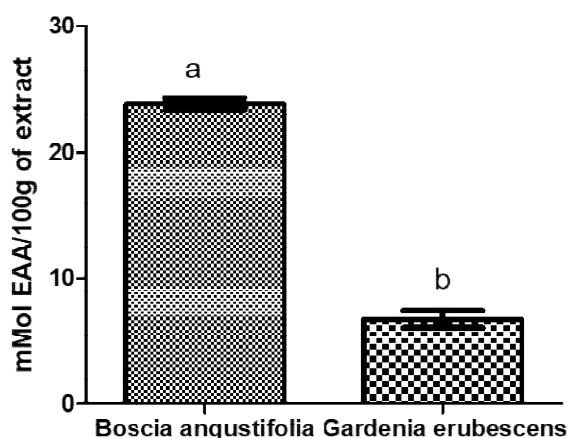


Fig. 4. Results of the reduction of the iron ion

The values are represented on average \pm Standard deviation ($n = 4$). The letters (a-b) are significantly different at $P < 0.05$

Table 1. Inhibition diameters of extracts (20 μ g / mL) according to microbial strains

Extract	Diameter (mm)				
	<i>S. dysenteria</i>	<i>S. aureus</i>	<i>S. thyphi</i>	<i>B. cereus</i>	<i>E. coli</i>
<i>Boscia angustifolia</i>	10 \pm 1.00 ^a	10 \pm 0.1 ^{a a}	9 \pm 0.05 ^a	14 \pm 0.12 ^a	9 \pm 0.00 ^a
<i>Gardenia erubescens</i>	6 \pm 0.09 ^{a a}	6 \pm 0.07 ^{a b}	6 \pm 0.15 ^b	6 \pm 0.13 ^b	6 \pm 0.06 ^b

S. dysenteria: *Shigella dysenteria*; *S. aureus*: *Staphylococcus aureus*; *S. thyphi*: *Salmonella thyphi*; *B. cereus*: *Bacillus cereus*; *E. coli*: *Escherichia coli*

The values are represented on average \pm Standard deviation ($n = 3$). The letters (a-b) are significantly different at $P < 0.05$

Gardenia erubescens trunk bark was relatively less sensitive to most of the strains used (Table 1).

4. DISCUSSION

The determination of total phenolics in the extracts revealed the presence of these chemical groups. Work done by Nacoulma and

collorateurs [5] had highlighted the important presence of terpenic compounds in the bark of *Gardenia erubescens* [18]. The results on the determination of total flavonoids in the ethanolic extract of *Gardenia erubescens* are supported by some authors who had found that the different parts of this plant and especially the fruits were overflowing with large quantities of flavonoids (298.50 \pm 9.25 mg EQ / 100 g of extract) [19].



Photo 3. Results of inhibition diameters of *Boscia angustifolia* on *Staphylococcus aureus* (A) and *Salmonella thyphi* (B)

S. aureus: *Staphylococcus aureus*; *S. thyphi*: *Salmonella thyphi*

The ethanolic extract of *Boscia angustifolia* had a relatively lower flavonoid content than the ethanolic extract of *Gardenia erubescens* trunk bark. These results confirm the characterization tests carried out on different parts of *Boscia angustifolia* where there was a lack or low content of flavonoids [20].

Antioxidant tests revealed that the *Boscia angustifolia* trunk bark extract had a better ability to reduce the DPPH radical. This antioxidant capacity of this extract could be justified by the phenolic compounds which are recognized according to the literature to possess an antioxidant potential [21]. The ability to reduce iron ion by the extracts would highlight a provision to give electrons or protons to stabilize the biomembranes [22]. This principle would allow these extracts to trap released free radicals following the aggressions of the body or to protect the body against possible aggressors [23].

Boscia angustifolia also showed antimicrobial activity. Plant extracts from the Caparaceae family are known to have good antimicrobial activity [24]. In addition, the ethanolic extract of *Boscia angustifolia* leaves has a translucent appearance that would highlight the presence of essential oil. In the literature, essential oils are endowed with antimicrobial potential [25,26]. The best inhibition diameter of the extracts was observed in *Bacillus cereus*. This microorganism is believed to be responsible for certain diseases in the human body, such as the types of

infections characterized by diarrheal symptoms [27]. Thus, the antioxidant capacity of the extracts corroborates the results of the antimicrobial tests, since the antioxidant power reduces the ability to move certain pathogenic microorganisms [28].

5. CONCLUSION

This study has added a scientific basis on medicinal plants in a general way and specifically on *Boscia angustifolia* and *Gardenia erubescens*. Phytochemistry has revealed phenolic compounds and flavonoids in the extracts. The biological activities of the extracts through their antioxidant and antimicrobial properties have been highlighted in this work. The therapeutic virtues of these two plants could justify their use in traditional medicine. These plants deserve to be valued for much more efficient use by the local population. Thus they could be a source for in-depth studies in the isolation of active ingredients against certain diseases related to oxidative stress and certain pathogenic microorganisms.

CONSENT

All authors declare that "written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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