

Original Research Article

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)

ABSTRACT

Aims: *Boscia angustifolia* 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.

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~~ 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* 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,

Comment [DM1]: Once the full scientific name is written, please write the abbreviated form thereafter

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* 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. MATERIAL AND METHODS

2.1. Plant material

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.

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 µL 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. Antibiomicrial extracts test

2.7.1 Microbial strains

Five bacterial strains including: 3 Gram-negative strains (*Escherichia coli* ATCC8739, *Salmonella typhi*, *Shigella dysenteria* 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 method

The diameter of the inhibition zone of the extracts was determined by the well method [15]. A volume of 10 µL of extract (20 µg / 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 ± 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

Figure 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 ± 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.

Comment [DM2]: Italics

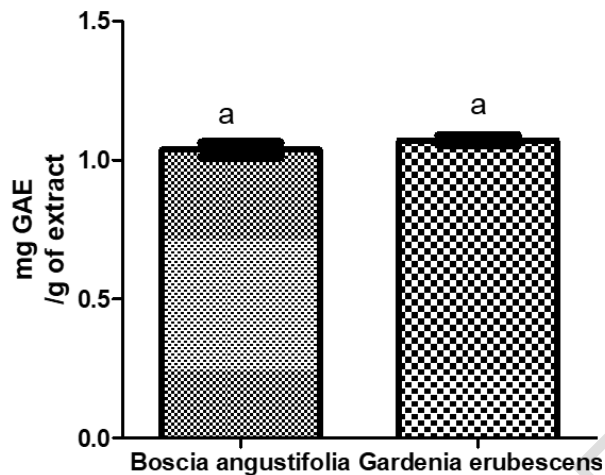


Fig. 1. Results of the determination of total phenolics
 The values are represented on average \pm Standard deviation ($n = 4$).

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 ± 0.001 mg QE / g extract (fig. 2).

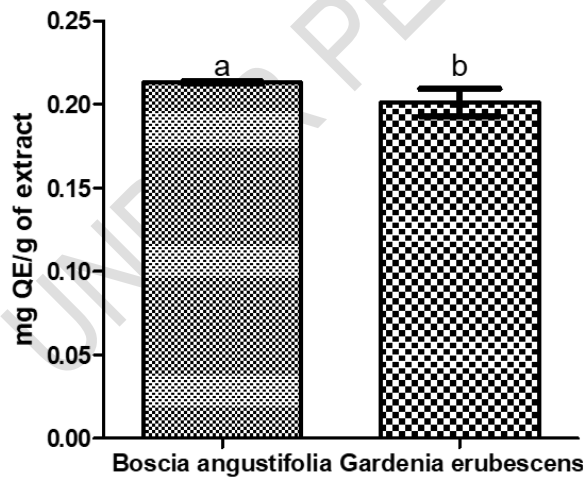


Fig. 2. Results of the determination of total flavonoids
 The values are represented on average \pm Standard deviation ($n = 4$). The letters (a-b) are significantly different at $P < 0.05$.

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).

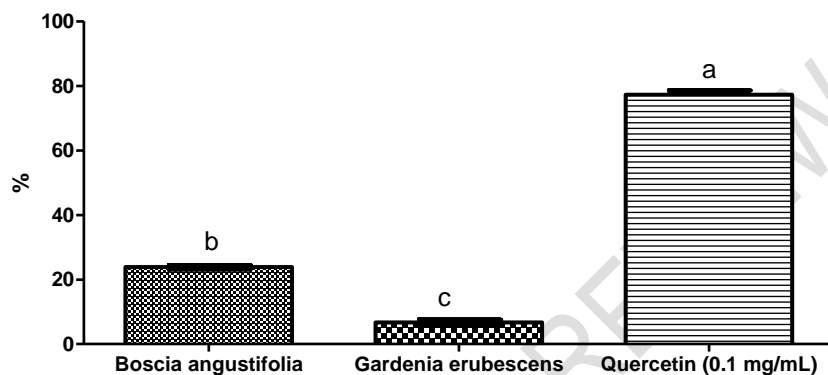


Fig. 3. Results of the determination of anti-radical activity DPPH*

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

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 / 100g extract) (fig. 4).

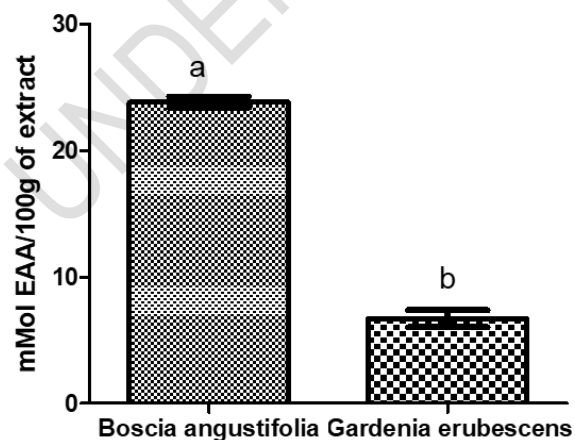


Fig. 4. Results of the reduction of the iron ion

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

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. In contrast to this extract, the ethanolic extract of *Gardenia erubescens* trunk bark was relatively less sensitive to most of the strains used (Table 1).

Table 1: Inhibition Diameters of Extracts According to Microbial Strains

Extract	Diameter (mm)				
	<i>Shigella d</i>	<i>Staphylococcus a</i>	<i>Salmonella t</i>	<i>Bacillus c</i>	<i>Escherichia c</i>
<i>Boscia angustifolia</i>	10 \pm 1.00 ^a	10 \pm 0.1 ^{aa}	9 \pm 0.05 ^a	14 \pm 0.12 ^a	9 \pm 0.00 ^a
<i>Gardenia erubescens</i>	6 \pm 0.09 ^{aa}	6 \pm 0.07 ^{ab}	6 \pm 0.15 ^b	6 \pm 0.13 ^b	6 \pm 0.06 ^b

Shigella d: *Shigella dysenteriae*; *Staphylococcus a*: *Staphylococcus aureus*; *Salmonella t*: *Salmonella thyphi*; *Bacillus c*: *Bacillus cereus*; *Escherichia c*: *Escherichia coli*

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

4. DISCUSSION

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

Comment [DM3]: Provide information on minimum inhibitory concentration (MIC).

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 a 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 (WHERE EVER APPLICABLE)

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."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

REFERENCES

1. Sarr O, Bakhom A, Diatta S, Akpo LE. L ' arbre en milieu soudano-sahélien dans le bassin arachidier (Centre-Sénégal). J Appl Biosci. 2013;61(ISSN 1997–590):4515–29.
2. Malgras D (R. P. Arbres et arbustes guérisseurs des savanes maliennes. Editions K. 22 - 24, boulevard Arago, 75013 Paris; 1992. 480 p.
3. N'do JY, Hilou A, Ouedraogo N, Sombié EN, Traoré TK. Phytochemistry, Antioxidant, and Hepatoprotective Potential of *Acanthospermum hispidum* DC Extracts against Diethylnitrosamine-Induced Hepatotoxicity in Rats. *medicines*. 2018;42(5):1–13.
4. Keita A, Kiniffo H V, Nseyya AM, Saadou M, Souza S De, Tchabi A, et al. et pharmacopée contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Agence de. Paris; 1989. 895 p.
5. Nacoulma OG. Plantes médicinales et Pratiques médicinales Traditionnelles au BURKINA. Université de Ouagadougou; 1996.
6. Jurisch K, Hahn K, Wittig R, Bernhardt-Römermann M. Population Structure of Woody Plants in Relation to Land Use in a Semi-arid Savanna, West Africa. *Biotropica*. 2012;0(0):1–8.
7. Tarry DW. Observations on the ecology of *Glossina morsitans morsitans* Newst. in the Guinea-Sudan transition savanna of Northern Nigeria. *Ann Trop Med Parasitol*. 2016;4983:9.

Comment [DM4]: References are not uniform and not according to the journal's instructions

8. Kerharo J, Adam JG. La Pharmacopée sénégalaise Traditionnelle Plantes médicinales et Toxiques. Vigot Frère. Paris; 1974. 1011 p.
9. N'do JY, Tibiri A, Sombie EN, Tata TK, Ouédraogo N, Hilou A, et al. Ethnobotany and preliminary bioactivity investigation on hepatoprotective medicinal plants from the Mouhoun Region of Burkina Faso. *Int J Phytomedicine*. 2018;10(2):73–80.
10. Singleton VL, Orthofer R, Lamuela R, Rosa M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. P Lester (Ed), *Methods Enzymol*. 1999;299:152–78.
11. Ouattara MB, Kiendrébéogo M, Konaté K, Compaoré M, Meda RN, Bationa JH, et al. Antibacterial Potential and Antioxidant Activity of Polyphenols of *Sesbania pachycarpa*. *African J Sci Res*. 2011;5, No. 1:17.
12. Alisi CS, Ojiako O a, Osuagwu CG, Onyeze GOC. Free Radical Scavenging and In-vitro Antioxidant Effects of Ethanol Extract of the Medicinal Herb *Chromolaena odorata* Linn . *Br J Pharm Res*. 2011;1(4):141–55.
13. Hinneburg I, Dorman D, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem*. 2006;97(1):122–9.
14. Lennette HE, Bellows A, Hausler JW, Shadomy HJ. *Manual of Clinical microbiology*. Man Clin Microbiol 4th. 1987;ID: 971432(ISBN-13: 978-0914826699.):336–59.
15. Mihin HB, Somda MK, Kabore D, Souleymane Sanon AYA, S.Traore A, Ouattara AS. Biopreservation of Meat by Using Antimicrobial Properties of Essential Oil from *Laggera Aurita* in Burkina Faso. *Adv Nutr Food Sci*. 2019;Volume 201(Issue 02):12.
16. Rhayour K. Etude du mécanisme de l'action bactéricide des huiles essentielles sur *Escherichia coli*, *Bacillus subtilis* et sur *Mycobacterium phlei* et *Mycobacterium fortuitum*. Thèse de doctorat. Université Sidi Mohamed Ben Abdellah, Maroc; 2002.
17. Negreiros MO, Pawlowski A, Soares GLG, Motta AS, Frazzon AP. In vitro antimicrobial activity of essential oils from *Heterothalamus* Less. (Asteraceae) against clinically relevant bacterial and fungal species. *Brazilian J Biosci*. 2016;14(1):26–31.
18. Ojelere OO. Phytochemicals, proximate, mineral composition and antimicrobial activity of some selected medicinal plants seeds. University of Ibadan, Nigeria; 2014.
19. Lamien-Meda A, Lamien C, Compaoré M, Meda R, Kiendrebeogo M, Zeba B, et al. Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. *Molecules*. 2008;13:581–94.
20. Hassan SW, Umar RA, Lawal M, Bilbis LS, Muhammad BY, Dabai YU. Evaluation of antibacterial activity and phytochemical analysis of root extracts of *Boscia angustifolia*. *African J Biotechnol*. 2006;5(September):1602–7.
21. Batista R, Jesus A De, Júnior S, Oliveira AB De. Plant-Derived Antimalarial Agents: New Leads and Efficient Phytomedicines. Part II. Non-Alkaloidal Natural Products. *Molecules*. 2009;14:3037–72.

22. Methorst C, Huyghe E. Volume 24 -Septembre 2014 -Hors-série 3 Stress oxydant et infertilité masculine : physiopathologie et intérêt thérapeutique des antioxydants et les membres du Comité d'Andrologie et de Médecine Sexuelle de l'Association Française d'Urologie Sous-Comité Fe. Progrès en Urol [Internet]. 2014;24:4–10. Available from: <http://www.urofrance.org/sites/default/files/fileadmin/documents/data/PU/2014/00240HS3/4/index.pdf>
23. Vinothkumar KR, Henderson R. Structures of membrane proteins. Q Rev Biophys. 2010;43:65–158.
24. Sen S, Chakraborty R. The Role of Antioxidants in Human Health. Creative E. Inde; 2011. 37 p.
25. Thi N, Min J, Chul S. Chemical composition , antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb .) Merr and Perry buds. Food Chem Toxicol [Internet]. 2008;46(12):3632–9. Available from: <http://dx.doi.org/10.1016/j.fct.2008.09.013>
26. Hajlaoui H, Mighri H, Noumi E, Snoussi M, Trabelsi N, Ksouri R. Chemical composition and biological activities of Tunisian *Cuminum cyminum* L . essential oil : A high effectiveness against *Vibrio* spp . strains. Food Chem Toxicol [Internet]. 2010;48(8–9):2186–92. Available from: <http://dx.doi.org/10.1016/j.fct.2010.05.044>
27. Dromigny. *Bacillus cereus*. Collection « Monographies de microbiologie ». France: Éditions Lavoisier Paris; 2008. p. 4.
28. Compean KL, Ynalvez RA. Antimicrobial activity of plant secondary metabolites: a review. Res J Med plant. 2014;8(5):204–13.