Original Research Article

Antimicrobial activity and toxicity of the aerial parts extracts of *Richardia brasiliensis* collected in Southern-Benin.

ABSTRACT

Aims: the aim of this study is to evaluate the antimicrobial activity of the *r. Brasiliensis* aerial part extracts collected in southern-benin**.**

Methodology: The phytochemical screening was performed by a differential precipitation staining method. Antimicrobial activity was evaluated on *s. Aureus* strains isolated from skin infections and ten reference strains by the solid-medium diffusion method. The minimum inhibitory concentrations (mic) and minimum bactericidal concentrations (mbc) were determined by the liquid macro-dilution method. The cytotoxic effect of the extracts was evaluated on artemia salina larvae obtained by hatching.

Results: The extracts used are aqueous and ethanolic extracts with respective yields of 15.5% and 10.30%. The phytochemical screening showed a strong presence of tannins, flavonoids, terpenes, steroids and a medium presence of alkaloids, anthocyanins and mucilage's. The two extracts variously ($p<0.001$) inhibited the growth of *s. Aureus* strains isolated from skin infections and four reference strains (*staphylococcus aureus atcc29213, pseudomonas aeruginosa atcc27853, proteus mirabilis a24974, escherichia coli atcc25922*). However, there is no difference ($p > 0.05$) in inhibition of strains growth between 24h and 48h. The largest diameter (21±0.75mm) of inhibition with the reference strains was obtained with *p. Aeruginosa* by action of the aqueous extract. Regarding *s. Aureus* strains isolated from skin infections, the largest diameter of inhibition is about 19.25±2.75mm obtained with strains isolated from ulcers. The average mics of 2.81 mg/ml and 2.08 mg/ml were obtained respectively for the aqueous and ethanolic extracts in the presence of the reference strains. The lc50 determination obtained using the regression line is 0.36 mg/ml for the aqueous extract and 1.16 for the ethanolic extract.

Conclusion: The aqueous extract is more effective because of its action spectrum. This extract can be used for the development of a soap or ointment to fight against skin infections.

Keywords: Richardia brasiliensis, antimicrobial activity, skin infections, southern-Benin.

1. INTRODUCTION

Medicinal plants occupy an important place in the African pharmacopoeia [1]. According to the WHO [2], it is estimated that nearly 80% of the African population uses plants to treat themselves. Among the many species exploited is *Richardia brasiliensis*. Of Brazilian origin, the plant is used as an expectorant, antiemetic, and diaphoretic [3]. This plant has important

pharmacological activities in the treatment of diabetes, hemorrhoids, and skin diseases. Furthermore, in South Africa, ethnobotanical studies have reported that the plant is an effective traditional means of fighting candidiasis and fungal infections [4]. In Benin, this plant is used in the treatment of scabs that characterize certain skin diseases but it has never been the subject of a scientific study. This is therefore proof that traditional medicine still has unexplored potential. Is it necessary to remind that remedies as effective as quinine (leader of anti-malaria drugs), morphine (powerful analgesic), rye ergot (anti-migraine virtues), curare (myorelaxant properties) are of plant origin! [5].

Skin infections are the result of a microbial attack, whether it be a bacterium, a parasite or a fungus. Epidemiological statistics show that skin infections are very common in children and remain one of the main reasons for medical consultation [6]. They occur most often during the summer, a time when heat and humidity favor the growth of most microorganisms especially bacteria [7]. The main microbial skin infections are: impetigo, cellulitis, abscess, boil and folliculitis. The bacteria most involved in skin infections are *S. aureus, P. aeruginosa, E. coli and P. mirabilis* [8]. However, the hope that was born with the discovery of antibiotics has been dissipated with the emergence of microorganisms resistant to these drugs. The incidence and increasing frequency of microorganisms resistant to most antibiotics is growing [9]. The evolution of acquired bacterial resistance to antibiotics now results in high rates of multidrug resistance in some bacterial species that were multi-susceptible [10;11]. In 2008, antibiotic sensitivity tests of *S. aureus* strains were performed in a French study and showed that 86% of strains isolated from patients were resistant to penicillin and erythromycin [12]. This crucial phenomenon of antibiotic resistance leads to the continuous increase in the price of available drugs [13;14]. Today, essential generic drugs are no longer within the reach of populations because pharmaceutical companies have appropriated them. Faced with such situations, low-income populations have resolutely turned to the use of plants for their health problems.

However, the main problem of traditional treatments, especially those based on plants, is the lack of scientific knowledge regarding efficacy, mode of action, active ingredients, doses to be used, indications, lack of property, safety and quality control. This study is part of the research of new bioactive molecules through the valorization of *R. brasiliensis* from the traditional Beninese pharmacopoeia. Thus, the aim of this study is to evaluate the antimicrobial activity of the *R. brasiliensis* aerial part extracts collected in southern-Benin. It will also allow the discovery of new active ingredients such as antimicrobial agents. Moreover, the results of this study will contribute to the orientation of our populations towards the plant, thus offering them low-cost remedies.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Plant material

The plant material consists of the aerial part of *Richardia brasiliensis*. The collect were made on April 23 and 30, 2021 at the University of Abomey-Calavi and Dékoungbé in the town of Abomey-Calavi. They were then identified and certified at the National Herbarium of Benin under the number YH 610/HNB. After drying at laboratory temperature (16°C), they were ground into powder using a Retsch grinder type SM 2000/1430/Upm/Smf and stored in jars in the laboratory.

2.1.2. Microbial strains

The microorganisms tested include both bacteria (Gram positive and, negative) and champions. These microorganisms are composed of ten (10) reference strains (*Escherichia coli ATCC25922, Pseudomonas aeruginosa ATCC27853, Proteus mirabilis A24974, Micrococcus luteus, Staphylococcus epidermidis T22695S, Escherichia coli 0157: H7ATCC, Streptococcus oralis, Enterococcus faecalis, Candida albicans, Staphylococcus aureus ATCC29213*) and 9 strains of *Staphylococcus aureus*. These 9 strains of *S. aureus* are part of the collection of the Laboratory of Biology and Molecular Typing in Microbiology and come from various types of skin infections: pus from boils, abscesses and ulcers.

2.2. Methods

2.2.1. Preliminary phytochemical screening

The preliminary phytochemical screening was performed directly on the plant powder. It is a qualitative analysis based on differential staining and precipitation reactions. The method used is that of [15] adapted to the conditions of the National Pharmacognosy Laboratory (LNP).

2.2.2. Getting the extracts

Two types of extracts were prepared. These are ethanolic and aqueous extracts. The choice of these types of extracts is based on the way the plant is traditionally used.

2.2.2.1. Aqueous extracts

The total aqueous extracts were obtained using the method developed by [16]. For this purpose, 50 g of dry plant powder were macerated in 500 ml of distilled water on a mechanical shaker for 48 hours at room temperature. The homogenate obtained was filtered twice on absorbent cotton. This filtrate was then dried at 50°C in the oven and the powder thus obtained constitutes the total aqueous extract ready to be used.

2.2.2.2. Ethanolic extracts

The extraction method used is inspired by the protocol used in the work of [17;18]. This method allows to put in contact all the phases of the powder with the solvent thanks to the agitation. In practice, 50 g of dry plant powder was macerated in 500 ml of 96% ethanol under continuous stirring for 48 hours. After two successive filtrations on absorbent cotton, the filtrate was concentrated in rotavapor under vacuum at the temperature of 50°C. After concentration, the filtrates were deposited in the oven at 50°C until a dry mass was obtained. The powders obtained after these extractions were immediately used for the biological tests, otherwise they were kept at 4°C in sterile glass bottles.

2.2.2.1. Extraction yield

The extraction yield is defined as the ratio between the mass of dry extract obtained and the mass of plant material processed [19]. It was obtained according to the following formula:

$$R(\%) = (Me/ Mv) \times 100$$

R (%) : Yield in %,Me : Mass of dry extract, Mv : Mass of plant material used.

2.2.3. Antibacterial activity of *Richardia brasiliensis* extracts

2.2.3.1. Sensitivity test

The *in vitro* antibacterial activity of the different extracts obtained was demonstrated by the Muller Hinton (MH) solid medium diffusion method with the use of paper (Whatman N°1) in the form of a disc soaked with extract [20]. A bacterial pre-culture (1 colony in 1 ml of liquid Mueller-Hinton) from the previous day is diluted to obtain a turbidity of 0.5 on the McFarland scale (10^8 CFU/ml) and reduced to 10^6 CFU/ml in sterile distilled water. This bacterial suspension (1000 µl) is used to flood a petri dish containing Mueller-Hinton agar (Bio Rad, France). Using a perforator, the 6 mm diameter disc papers were made. The sterile discs are deposited, under aseptic conditions, on plates once flooded with bacterial culture. On the deposited discs, 30 µl of extract to be tested is inoculated under aseptic conditions. For each extract, the experiment is duplicated and a negative control is performed with the solvent instead of the extract. The plates are then left for 15-30 min at room temperature before incubation at 37 °C in the oven for 24 h [21] and 48 h. Inhibition diameters are measured using a graduated ruler [22] after incubation times of 24 h and 48 h.

2.3.3.1. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined in the present work by the macrodilution method in liquid medium [23] with visual appreciation of the growth of microorganisms. In the absence of 48-well plates (6 rows of 8 wells), test tubes were used. To do this, starting from a stock concentration of 20 mg/ml of sterile extract, a concentration range was performed through dilutions of ½ reason. Thus, starting from 9 tubes (T0 to T8), 1 ml of sterile distilled water was distributed in all tubes except the first one (T0). To carry out the concentration range, 1ml of plant extract of concentration 20 mg/ml were deposited in the T0 tube. Then, 1ml of plant extract of concentration 20mg/ml were also added to the contents of tube T1. After homogenization, 1ml of the mixture (extract + distilled water) from tube T1 was taken and transferred to tube T2. Then, 1ml taken from tube T2 was transferred to tube T3. This operation was continued until tube T6 from which 1ml was discarded. At the end of this series of dilutions we obtained a series of concentrations varying from 100 to 0.15625 mg/ml in tubes T0 and T7. Tube T8 received only sterile distilled water to serve as a positive growth control tube. An inoculum of each bacterial strain with turbidity adjusted to 0.5 on the Mc Farland scale was prepared in MH broth. 1ml of inoculum was added to each tube (T0 to T8). After 24 h of incubation, bacterial growth which is reflected by turbidity was examined in each tube. The MIC of an extract towards a given strain corresponds to the smallest of the concentrations showing no growth visible to the naked eye.

2.3.3.2. Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined based on the results of the MIC determination. To do this, after identifying the MIC, we used a loop to inoculate all the other tubes from the MIC to the high concentrations on petri dishes containing MH agar medium. The plates were examined after 24 h of incubation at 37°C. Upon observation, the lowest concentration of the extract at which no bacterial growth is observed corresponds to the BMC [24].

2.3.4. Test of the cytotoxic activity of the different extracts

The cytotoxic effect of the extracts was evaluated following an adaptation of the method described by [25]. The tests were performed on larvae obtained by hatching 10 mg of Artemia salina eggs (ARTEMIO JBL GmbH D-67141 Neuhofem) under continuous agitation in 1l seawater for 72 h. To 1 ml of each geometric serial dilution of reason ½ of extract prepared from a stock solution of 20 mg/ml, in seawater, was added 1 ml of seawater containing 16 larvae. The number of surviving larvae was counted after 24 h of incubation. The LD50 was determined from the regression line obtained from the curve representing the number of surviving larvae as a function of extract concentration.

2.3.5. **Data analysis**

Data entry and analysis was done in Excel 2013 Spreadsheet. Data analyses were done using GraphPad Prism 8 software. For each extract, the lethal concentration that causes 50% larval death (LC50) was calculated with a 95% confidence interval by linear regression analysis and also using the probit analysis method following. A regression line equation, obtained from the larval mortality curve, is used to calculate the concentration (LC50) corresponding to the death of half the larvae. The degree of leaf toxicity was evaluated based on the correspondence table (Table I) established by Mousseux [26].

Table I: Correspondence between LC50 and cytotoxicity

LC50	Cytotoxicity
LC50 ≥ 0,1mg/ml	- (No toxicity)
0.1 mg/ml >LC50 ≥ 0.050mg/ml	+ (low cytotoxicity)
0.050 mg/ml > LC50 ≥ 0.01mg/ml	+ + (average cytotoxicity)
LC50 < 0.01mg/ml	+ + + (high cytotoxicity)

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Phytochemical screening

Table II shows the result of the phytochemical screening performed with the powders of the studied plant. There is a strong presence of gall tannins, catechic tannins, flavonoids and a medium presence of Anthocyanins, Terpenes, Steroids, Alkaloids and Mucilages. However, the plant does not contain Leucoanthocyanins, saponosides, coumarins, reducing compounds, quinones, cyanogenic derivatives, O-heterosides, reduced genine O-heterosides and C-heterosides.

Table II: Results of phytochemical screening

Secondary metabolites	Results
Gallic tannins	+++
Catechic tannins	+++
Flavonoids	+++
Anthocyanin	++
Leucoanthocyanins	-
Terpenes	++
Steroids	++
Saponosides	-

Coumarin	-
reducing compounds	-
Quinones	-
Cyanogenic derivatives	-
Alkaloids	++
Mucilage	++
O-Heterosides	-
C-Heterosides	-
Cardiotonic heterosides -	-

Legend: +++: Strong presence; ++ : Average presence ; + : Low presence ; - : Absence

3.1.2. Extraction yield

Analysis of the extraction yield with the two solvents (Figure 1) showed that the extraction yields of the water extracts (15.50%) were higher than those of the ethanol extracts (10.30 %). Water therefore concentrated the secondary metabolites better compared to ethanol.

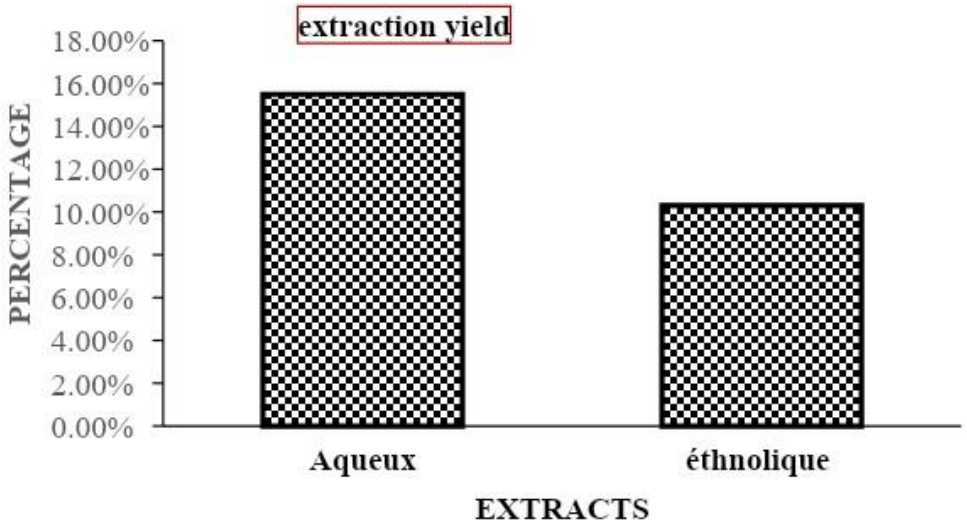

Figure 1: Yield of the prepared extracts

3.1.3. Antibacterial activity

The reference strains showed a variable sensitivity in the presence of ethanolic and aqueous extracts of *Richardia brasiliensis*. We notice that 3/10 showed sensitivity to ethanolic extracts and 4/10 were sensitive to aqueous extract (Table III). The highest inhibition diameter (21±0.75 mm) was obtained with the aqueous extract against *Pseudomonas aeruginosa* strain ATCC27853 while the lowest inhibition diameter (12 ±2.00 mm) was obtained with the ethanolic extract against the same pathogenic strain (Table III). The inhibition diameters varied between species but remained stable over time (Table III). There was a significant difference in the action of these two extracts ($p<0.001$).

Figures 2 and 3 show the inhibitory activity of the ethanolic and aqueous extracts of *Richardia brasiliensis* towards the reference strains as a function of time. It appears that the

extracts inhibit the proliferation of most of the pathogenic bacteria without any remanence over time. There is a very significant difference between the effect of the aqueous extracts on the reference strains ($p < 0.001$), but not as a function of time ($p > 0.05$). There is also a highly significant difference between the effect of ethanolic extracts on reference strains ($p < 0.001$), but not as functions of time ($p > 0.05$).

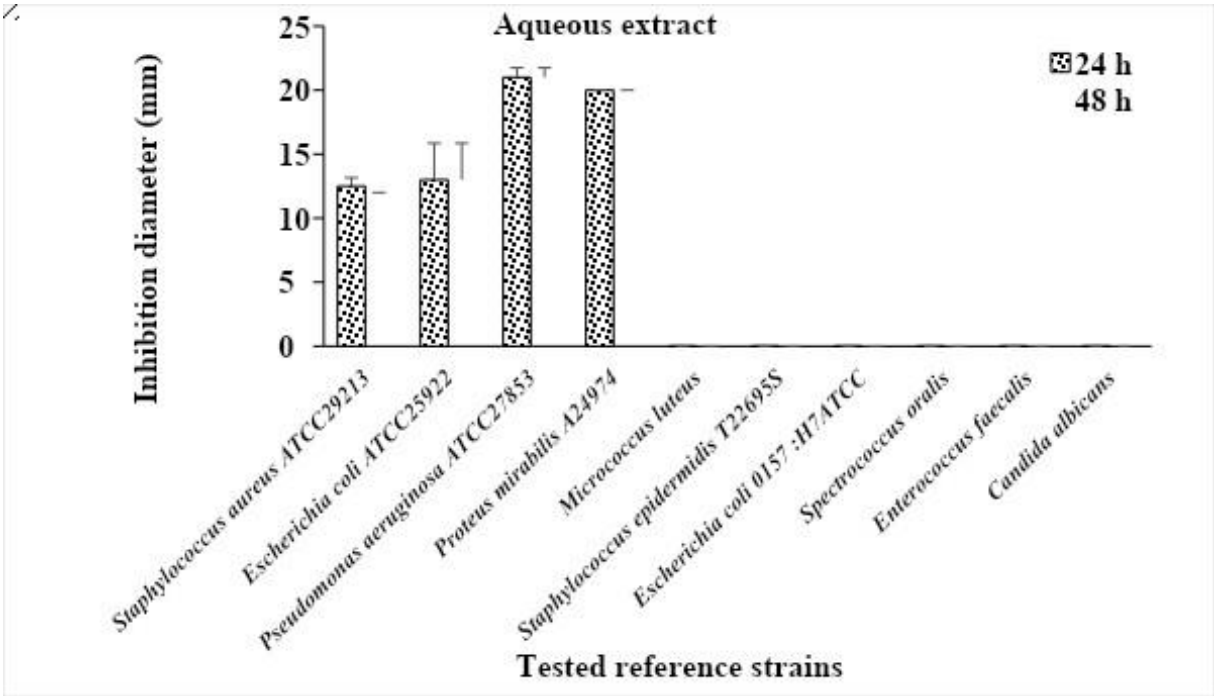

Figure 2: Inhibitory activity of *Richardia brasiliensis* aqueous extract against reference strains as a function of time.

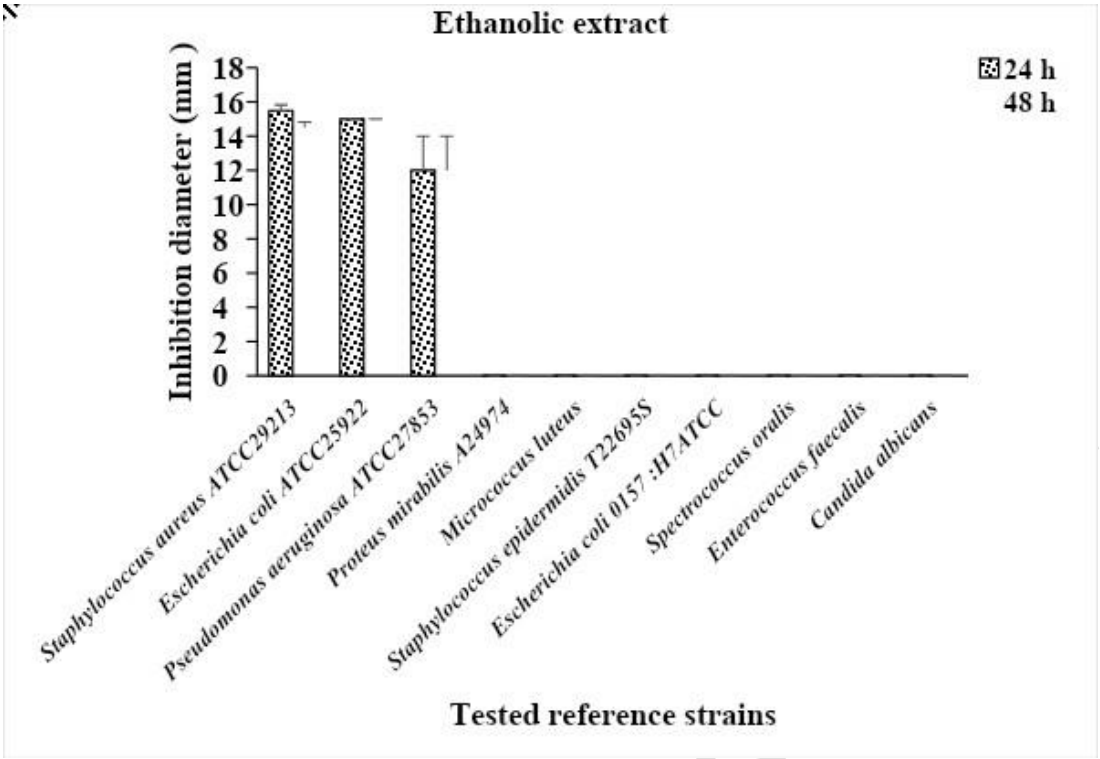

Figure 3: Inhibitory activity of ethanolic extracts of *Richardia brasiliensis* against reference strains.

Table III: Inhibitory activity of *Richardia brasiliensis* extracts against reference strains.

Strains	Inhibitory diameter in mm			
	Aqueous extracts		Ethanolic extracts	
	24h	48h	24h	48h
Staphylococcus aureus ATCC29213	12,5±0,66	12±0,00	15,5±0,33	14,5±0,33
Escherichia coli ATCC25922	13±2,86	13±2,86	15±0,00	15±0,00
Pseudomonas aeruginosa ATCC27853	21±0,75	21±0,75	12±2,00	12±2,00
Proteus mirabilis A24974	20±0,00	20±0,00	-	-
Micrococcus luteus	-	-	-	-
Staphylococcus epidermidis T22695S	-	-	-	-
Escherichia coli 0157:H7ATCC	-	-	-	-
Spectrococcus oralis	-	-	-	-
Enterococcus faecalis	-	-	-	-
Candida albicans	-	-	-	-

Figure 4 shows the inhibitory activity of *Richardia brasiliensis* extracts towards strains isolated from skin infections, we notice that the diameters of the inhibition zones in 24 hours and 48 hours are similar and this whatever the type of sampling and the type of extract. Thus, the difference in diameters taken at 24 hours is statistically similar to that taken at 48

hours (p>0.05). The highest mean inhibition diameter (19.25 mm) was obtained with the ethanolic and aqueous extracts against the *S. aureus* strain isolated from ulcers while the lowest mean inhibition diameter (12 mm) was obtained with the aqueous extract against the pathogenic *S. aureus* strain isolated from pus.

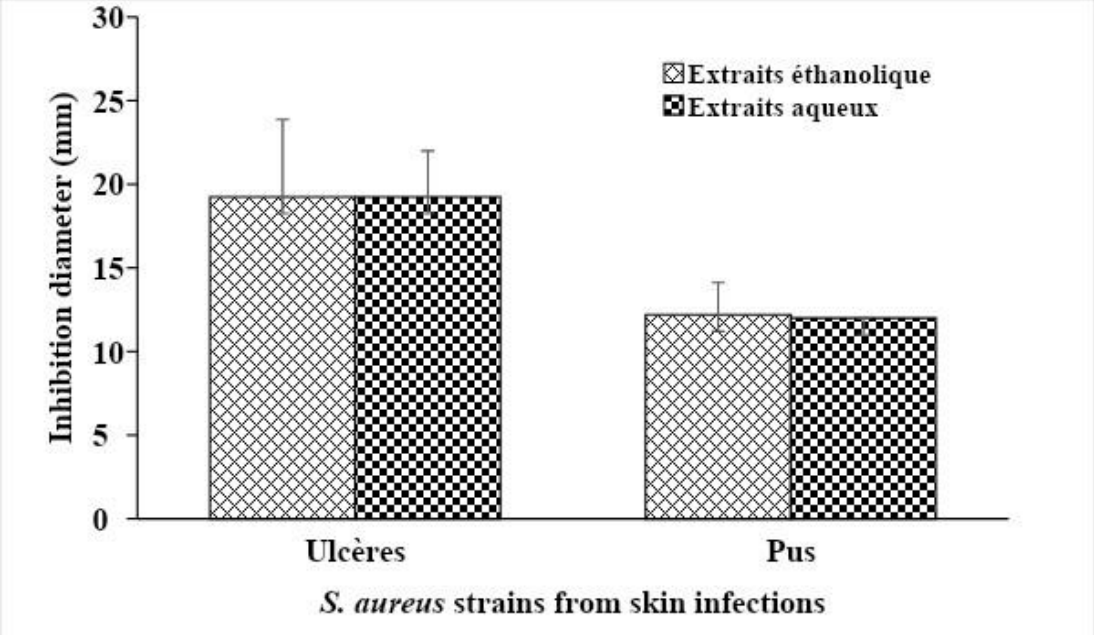

Figure 4: Comparative actions of extracts of *Richardia brasiliensis* on *S. aureus* strains from skin infections.

3.1.3.1. Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentrations obtained are variable according to the types of strains and the type of extract (Table IV and V). However, a low inhibitory concentration (1.25 mg /ml) was obtained using the aqueous and ethanolic extracts respectively for *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. For *S. aureus strains* isolated from skin infections, the lowest MIC was observed with *S. aureus* strains isolated from ulcers giving averages of 1.88±0.63 mg/ml (ethanolic extract).

Table IV: Minimum inhibitory concentrations of the two types of extracts on the reference strains tested.

Reference strains	Minimum Inhibitory Concentration (mg/ml)	
	Aqueous extract	Alcoholic extract
Staphylococcus aureus ATCC29213	2,5	2,5
Escherichia coli ATCC25922	1,2	2,5
Pseudomonas aeruginosa ATCC27853	5	1,25
Proteus mirabilis A24974	2,5	-

Table V: Minimum Inhibitory Concentrations of the two types of extracts on *S. aureus* strains from skin infections.

Origin of strains	Minimum Inhibitory Concentration (mg/ml)	
	Aqueous extract *	Alcoholic extract *
Pus	2,5**±0,00**	2,5**±0,00**
Ulcers	3,44**±1,56**	1,88**±0,63**
Average	2,97 **±**0,47	2.19**±0,315**

3.1.3.2. Determination of the Minimum Bactericidal Concentration (MBC)

After the determination of the MIC, the tubes without turbidities were plated on solid CBM in petri dishes. The results obtained 24 hours later show a slight growth of the strains even at a concentration of 10 mg/ml of aqueous and ethanolic extract.

3.1.3.3. Cytotoxicity of Richardia brasiliensis aerial part on shrimp larvae

The cytotoxic activity of *Richardia brasiliensis* leaf extract was evaluated with shrimp larvae (Artemia salina). In this shrimp larvae lethality assay, the percentages of mortality gradually increased with increasing concentration of the tested samples (Figure 5 and 6). The extracts of the aerial part of *Richardia brasiliensis* studied showed positive results (lethality) on the brine shrimp larvae Artemia salina indicating that the samples are biologically active. The LC50 determination obtained using the regression line is 0.36 mg/ml for the aqueous extract and 1.16 for the ethanolic extract.

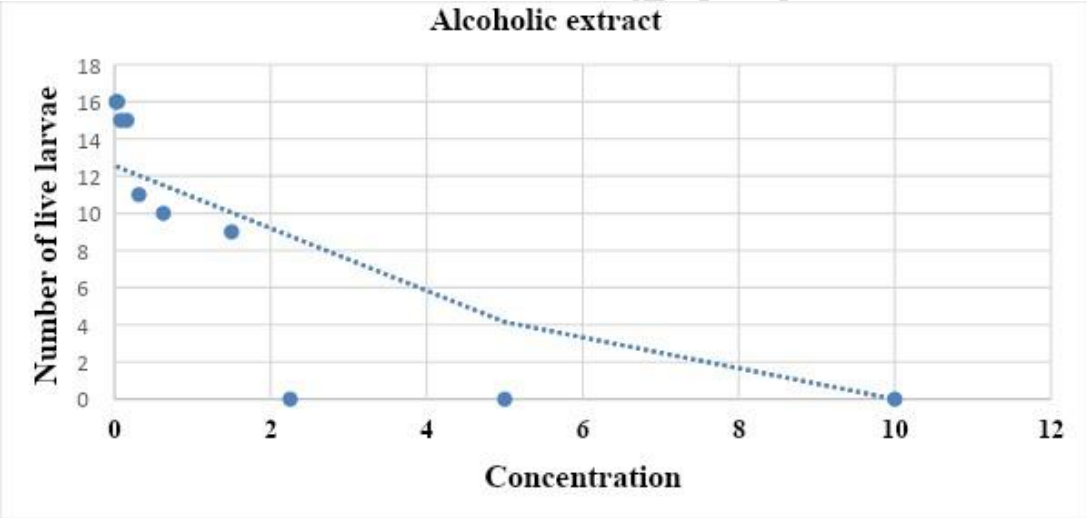

Figure 5: Cytotoxicity curve of ethanolic extract of *Richardia brasiliensis* on shrimp larvae

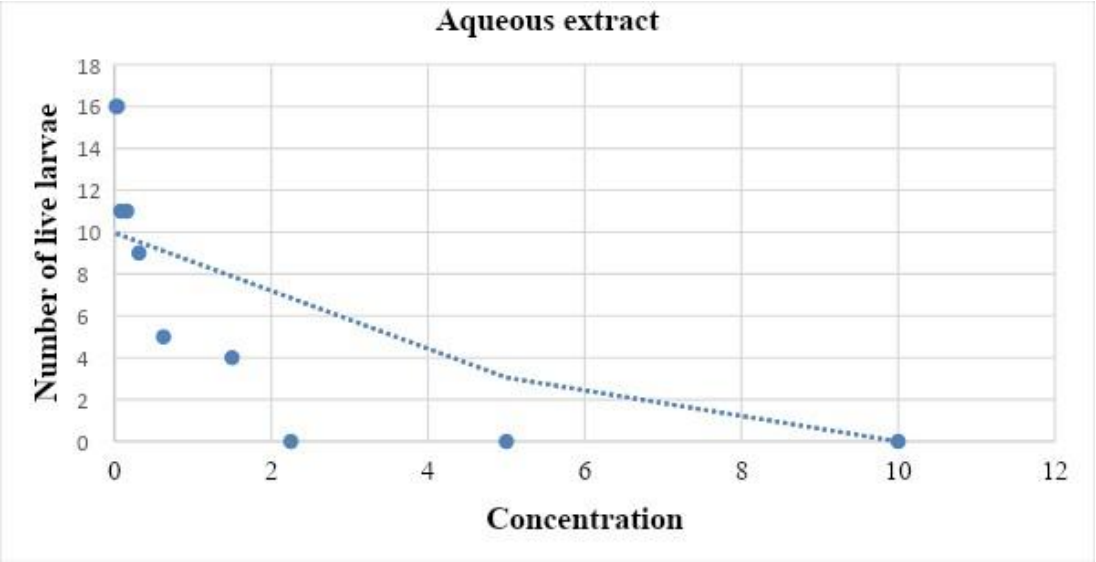

Figure 6: Cytotoxicity curve of aqueous extracts of *Richardia brasiliensis* on shrimp larvae

3.2. Discussion

In this study, the assessment of the antimicrobial activity of *Richardia brasiliensis* from the Beninese pharmacopoeia was performed on germs responsible for skin infections and on reference strains.

The phytochemical screening performed with the *Richardia brasiliensis* powders studied shows the presence of the secondary metabolites with antimicrobial properties sought. The studied plant is rich in phytochemical compounds. These leafy stems contain gallic and catechic tannins, terpenes and steroids, anthocyanins, mucilages, flavonoids and alkaloids. We note the absence of cyanogenic derivatives and cardiotonic heterosides, which are toxic substances that would call into question its health safety. The results obtained are similar to some [27; 3] who in addition to the metabolites found in this study also found coumarins and resins. The presence of a chemical family or not within the same species in different study areas, may be due to the influence of several factors such as variation in genetic makeup, weather conditions, geographical location of the plants, part of the plant studied and the extraction method used previously [28-30].

From the analysis of Figure 2, it can be seen that the aqueous extract gave the best yield (15.5%) compared to the 10.30% obtained for the ethanolic extract. These results corroborate those of Pinto et al. [31] who obtained a better yield with the aqueous extract (12.26%) compared to the ethanolic extract (7.76%). This could be explained by the fact that water better concentrated the secondary metabolites present in *Richardia brasiliensis* compared to ethanol.

The aqueous fraction of *Richardia Brasiliensis* exhibited stronger antibacterial activity than the ethanolic extract with an expanded spectrum of action towards *P. mirabilis* (Figure 2 and 3). These results are contrary to those of Hamid and Aiyelaagbe [32] who had shown that alcoholic extract of *Alafia barteri* better inhibits the in vitro growth of various bacterial strains (*S. aureus, E. coli, Pseudomonas aeruginosa and Candida albicans*) at concentrations ranging from 25-200 mg/ml. Karou et al. [33] reported that the sensitivity of bacteria to plant extracts based on the diameters of the inhibition zones is a function of the strain and bacterial species. In our study, 5 strains isolated from skin infections were sensitive to

ethanolic extract with mean inhibition diameters ranging from 6.5±6.72 mm to 19.25±4.63 mm. The presence of alkaloids, tannins, terpenoids and flavonoids may explain the antimicrobial property found in this study. Indeed, these secondary metabolites have been mostly recognized as the basis of antibacterial properties of medicinal plants [34-35]. Parida et al. [36] showed that in addition to these metabolites, steroids exhibit antibiotic activity. In addition, it has been shown that polyphenolic compounds in plants have antibacterial activity. The results of these works give us the probable origin of the good antimicrobial activity observed in the present study.

The Minimum Inhibitory Concentrations (MICs) obtained are variable depending on the type of strains and the type of extract (Table IV and V). In our study, the MICs are between 1.25 and 5 mg/ml for the reference strains tested, whereas for strains isolated from skin infections the mean MICs are between 0.9375±0.94 and 2.97 ±0.47 mg/ml. These values are largely lower than those of Mohammadi et al. [37] who had found MIC values between 62.5 and 250 mg/ml with the methanolic extract of *Euphorbia condylocarpa* root on the strains studied. Oikeh et al. [38] found MIC values between 12 µg/ml and 25 µg/ml on some Gram-negative bacteria using concentrated Juices from the fruits of the genus Citrus. On the other hand, Aibinu et al. [39] found higher values than ours (64≤ MIC≤ 256) using *Citrus aurantifolia* fruit extracts still on Gram negative bacteria. The differences observed between our MIC values and those of the above-mentioned authors could be explained by the extraction method, the solvents used and the plant organ. Thus, depending on the extraction method, the solvent used and even the plant organ, the antimicrobial active ingredients will not have the same concentrations in the extracts. In addition, depending on the location and the nature of the soil or climate, plants may have different "chemical profiles" [1]. Regarding the Minimum Bactericidal Concentrations (MBC), after 24 hours a slight growth of the seeded strains is observed for a concentration of 10 mg/ml. This shows that the ethanolic and aqueous noise extracts of *Richardia brasiliensis* have a minimum bactericidal concentration above 10 mg/ml.

This study also allowed to evaluate possible risks to which the population could be exposed by using *Richardia brasiliensis* leaves. The sensitivity curve showed that the larval mortality increases with the concentration. Thus, *A. salina* shrimp larvae are sensitive to aqueous extracts of *Richardia brasiliensis* leaves, which indicates that the sample is biologically active with a lethal concentration of 50% dead larvae (LC50) equal to 0.36 mg/ml for the aqueous extract and 1.16 for the ethanolic extract, which are higher than 0.1 mg/ml, upper limit of cytotoxicity according to Mousseux [26]. We deduce that the leaves of *Richardia brasiliensis* are not toxic. This corroborates with the results of Carey et al. [40] who observed, with the extracts of the leaves of *Bambusa vulgaris*, no mortality of animals, nor symptoms associated with toxicity such as convulsions, diarrhea or diuretic disorders. Therefore, the medicinal and food use of the leaves does not present any risk of short- or long-term intoxication for the populations; but also, the use of the leaves does not present any risk of extinction or loss of diversity of the genetic resources of the studied species.

4. Conclusion

The present work allowed to highlight the antimicrobial properties of different extracts of *Richardia brasiliensis* on strains of microorganisms in particular bacteria responsible for skin infections (*S. aureus*, *P. aeruginosa*, *E. coli*, *P. mirabilis*). The results obtained showed that all the extracts have a bacteriostatic activity on germs even in low concentrations. The concentrations at which the extracts act and their broad spectrum of action on the germs studied would justify the traditional use of the plant in the treatment of skin infections in Benin and South Africa. The idea of manufacturing a soap based on effective extracts thus

finds its originality and remains a better way to facilitate the use of the active principles of the plant in the treatment of skin infections.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Badiaga M. Etude ethnobotanique, phytochimique et activités biologiques de *Nauclea latifolia* Smith, une plante médicinale africaine récoltée au Mali. 24 Avenu des Landais. 2011 ;(12) :30-1. French
2. Organisation mondiale de la santé. Stratégies de l'OMS pour la médecine traditionnelle pour 2002-2005. WHO/EDM/TRM, Genève. 2002 ; 65.
3. Morais EC, Lavouisier FBN, Jéssica LAL, Larissa SM, Alencar BFFL, Fernando G. et al. Evaluation of Antimicrobial and Modulatory activity of the extract of *Richardia brasiliensis* Gomes. Ind J Trad Knowledge. 2013 ; 12 (4): 619-22.
4. Ndivhaleni AM, Lyndy JM, Jacobus NE. The traditional use of plants to manage candidiasis and related infections in Venda, South Afr J Ethnopharmacology. 2015; (168): 364–72
5. Clément RP. Aux racines de la phytothérapie : entre tradition et modernité. Phytothérapie. 2005 ; 3 (4) : 171-75. French
6. Larquey MEM. Staphylococcal and spectrococcal skin infections in children. Perfectionnement en pédiatrie. (2018); 25-31.
7. Rasigade JP. Diagnostic bactériologique des infections cutanées. 2012 ; 34-46
8. Dhar DA. Les infections cutanées bactériennes. North Atlanta Dermatol. 2019;(14): 23-6
9. Adejuwon AO, Agbaje EO, Idika N. Antifungal and antibacterial activities of aqueous and methanolic root extracts of *Carica papaya* linn. Int Res J Microbiol. 2011; (2): 270-77.
10. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; (31): 247-56.
11. Chen CJ, Huang YC, Chiu CH, Su LH, Lin TY. Clinical features and genotyping analysis of community acquired methicillin-resistant *Staphylococcus aureus* infections in Taiwanese children. J Pediatric Infect Dis. 2005;(24): 40-5.
12. Bernard P, Jarlier V, Santerre-Henriksen A. (2008). Sensibilité aux antibiotiques de souches *Staphylococcus aureus* responsables d'infections cutanées communautaires. Annal Derm Vénéréol. 2008; 13-9. French
13. Soro D, Koné MW, Kamanzi AK. Évaluation de l'activité anti-bactérienne et antiradicale libres de quelques taxons bioactifs de Côte-d'Ivoire. Eur J Sci Res. 2010;(40): 307–17.

14. Bagré I, Bahi C, Ouattara K, et al.. Étude botanique et exploration de l'activité antifongique de *Morinda morindoides* (Baker) Milne-Redh sur la croissance in vitro de *Cryptococcus neoformans*. Phytothérapie. 2011;(9): 136–41. French
15. Houghton PJ, Raman A. Laboratory Handbook for fractionation of natural extracts. Pharmacognosy Research Laboratories, Department of Pharmacy, king's college, London. 1998; 212.
16. Guede-Guina F, Vangah-Mandah M, Bonga GM, de Souza C. Activité antibactérienne d'un extrait végétal contre les germes opportunistes au cours du SIDA. Rev Méd Pharm. 1995;(9): 13-9. French
17. Sanogo R, Diallo D, Diarra S, Ekoumou C, Bougoudogo F. Activité antibactérienne et antalgique de deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali. Mali Méd. 2006 ;(21):18-24. French
18. N'Guessan JD, Bidie AP, Lenta BN, Weniger B, Andre P, Guede-Guina F. In vitro assays for bioactivity-guided isolation of anti-Salmonella and antioxidant compounds in *Thonningia sanginea* flowers. Afr J Biotech. 2007 (6): 1685-89.
19. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall Int. (Ed). NY, 1998; 49–188.
20. Anani K, Hudson JB, de Souza LC, Akpagana K, Towe GHN, Amason JT, et al. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm Biol. 2000;(38): 40-5.
21. Adesokan AA, Akanji MA, Yakubu MT. Antibacterial potentials of aqueous extract of Enantia chlorantha stem bark. *African journal of biotechnology*, 2007, *6*(22): 2502-05
22. Doughari JH, Pukuma MS, De N. Antibacterial eff ects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on Salmonella typhi. Afr. J. of biotech. 2007;(6): 2212–15.
23. Delarras C. Microbiologie. 90 heures de travaux pratiques. Gaétan Morien Editeur. ISBN : 291074907X, 9782910749071, 1998 ; 169-78.
24. Moroh JLA, Bahi C, Dje K, Loukou YG, Guede-guina F. Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne-redheat (Rubiaceae) sur la croissance in-vitro des souches d'*Escherichia coli*. Bulletin de la Société Royale des Sciences de Liège. 2008; (77) : 44-61. French
25. Kawsar SMA, Huq E, Nahar N. Cytotoxicity assessment of the aerial part of *Macrotyloma uniflorum* Lim. Int J Pharmaceutics. 2008;(4):297-00.
26. Ullah et al. (2013)
27. Mousseux M. Test de toxicité sur les larves de *Artemia salina* et d'entretien d'un élevage de balanes, Rapport de stage de deuxième année. DEUST Aquaculture ; Centre Universitaire de Nouvelle-Calédonie, France. 1995.
28. Figueiredo ADL, Bustamante KGL, Soares ML, Pimenta FC, Bara MTF, Fiuza TS et al. A valiaçào da atividade antimicrobiana das partes aéreas e raizes de *Richardia brasiliensis* Gomez (Rubiaceae), Rev Ciênc Farm Bàsica Apl. 2009;(30):193-96.
29. Malik F, Hussain A, Sadiq G, Parveen A, Wajid S, Shafat R. Phytochemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals. Afr J Pharm. Pharmacol. 2012 ;6(9):613-19.
30. Sujana P, Sridhar TM, Josthna P, Naidu CV. Antibacterial activity and phytochemical analysis of *Mentha piperita* L. (Peppermint) – an important multipurpose medicinal plant. Am J Plant Sci. 2013; (4), 77–83.
31. Akhtar N., Ihsan-ul-Haq, Bushra M. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian rev. of Chemis. 2018;11(2): 1223-35.

32. Pinto DSACT, Tavares JF, Tenório-Souza FH, Dias CS, Braz-Filho R, Cunha EVL. Metabólitos secundários isolados de Richardia brasiliensis Gomes (Rubiaceae). Rev Bras Farmacogn. 2008 ;18(3):367-72
33. Hamid AA, Aiyelaagbe OO. Preliminary Phytochemical, Antibacterial and Antifungal Properties of Alafia barteri Stem Grown in Nigeria. Eur J Med Plant. 2011;(1): 26-32.
34. Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J et al. Antibacterial activity of alkaloids from *Sida acuta*. Afr J Biotech. 2006;5(2): 195-00.
35. Esimone CO, Ebebe IM, Chah KE, Onyeka CG. Comparative antibacterial eff ects of *Psidium guajava*. J Trop Med Plant. 2003;(4): 185- 89.
36. Adejumobi JA, Ogundiya MO, Kolapo KA, Okunade MB. Phytochemical composition and in vitro antimicrobial activity of *Anogeissus leiocarpus* on some common oral pathogens. J Med Plant Res. 2008; (2): 193-96.
37. Parida MM, Dash PK, Saxaena P, Jana AM, Rao PVL. Perspectives on antimicrobial activity of natural plant products. In: Sharma RK, Arora R, editors. Herbal Drugs-A Twenty First Century Perspective. New Delhi: Jaypee Brothers Medical Puplishers. 2006; 484-96.
38. Mohammadi S, Asgary V, Sadat Shandiz SA, Heidari E, Jozaghkar H. Antimicrobial activity of methanolic root extract of *Euphorbia Condylocarpa* against pathogenic bacteria. Adv Studies Biol. 2015; (7): 55-64.
39. Oikeh E, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of diff erent citrus juice concentrates. Food Sci Nutr. 2016;4(1): 103–09.
40. Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T. Evaluation of the antimicrobial properties of diff erent parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr J Tradit Complement Alternative Med. 2007; 4 (2): 185-90.
41. Carey WM, Dasi, Rao NV, Gottumukkala KM. Anti-inflammatory activity of methanolic extract of *Bambusa vulgaris* leaves. Int J Green Pharmacy 2009; 234-38, DOI:10.4103/0973-8258.56282.