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Preliminary screening of the antimicrobial activity of nine medicinal plant species from Burkina Faso

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Infectious diseases, including skin infections, have always been treated by plants and other natural products since ancient times. This study investigated the antimicrobial activity of nine medicinal plant species from Gonsé Forest and Bangreweogo Park, Burkina Faso on seven microbial strains involved in skin infections. Sixty-eight organic and aqueous extracts from leaves, stem and root barks of the nine plants were tested on five bacterial and two fungal American Type Culture Collection (ATCC) strains using the disk diffusion and microdilution methods. Twenty-eight extracts (41.17%) from eight of the plants differently inhibited bacteria and fungi, the exception being *Wissadula amplissima* specie. Gram-positive Cocci (GPC) were the most sensitive to the extracts. Eleven extracts had a Minimum Inhibitory Concentration (MIC) lower than 1 mg/ml (MIC < 1 mg/ml). The extracts of *Opilia celtidifolia* gave the largest zones of inhibition ranging from 21.67 to 24.33 mm at 100 to 300 mg/ml on GPC and *Candida albicans*. Its MIC varied from 0.03 to 0.06 mg/ml on *Streptococcus pyogenes*, and from 0.07 to 0.24 mg/ml on *Streptococcus agalactiae*. Ethanolic extracts (80 and 96%) of *Lawsonia inermis* had MIC ranging from 0.29 to 9.37 mg/ml on the sensitive strains. The variable inhibitory activity displayed by the plants confirmed in part their traditional use in the treatment of some bacterial and fungal skin infections.

Key words: Infectious diseases, inhibitory activity, plant extracts, minimal bactericidal concentration (MBC), minimum inhibitory concentration (MIC), skin infections.

INTRODUCTION

Infectious dermatoses, or skin infections, are a major public health problem in developing countries (Hay et al., 2014; Marks et al., 2019). In tropical regions, the prevalence of skin infections has increased significantly with the occurrence of HIV/AIDS in 1980 (Silva et al.,

2012). In Mali it has been reported that skin infections represent 55.10% of consultation causes among children in the dermatology department of the National Centre for Support of Illness Control in Bamako (Fofana et al., 2016). In Senegal, infectious dermatoses accounted for

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64.7% of dermatological emergencies in the dermatology department of the Thiès regional hospital center (Dione et al., 2018).

Skin infections are particularly caused by bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus pyogenes*, characterized by high antigenic and antibiotic potential. Moreover, these bacteria are associated with biofilm formation, factor of additional virulence in hostile environments (Fu et al., 2017). *S. aureus* and *P. aeruginosa* are frequently involved in the colonization of chronic wounds or severe burns (Tong et al., 2015), spongiotic or acantholytic dermatoses, especially when they are extensive and localized in folds (Morand and Morand, 2017). *S. aureus* is responsible for a variety of skin and mucosal infections, ranging from mild infection (impetigo and simple cellulitis) (Tong et al., 2015) to complication (acute staphylococcal epidermolysis or Staphylococcal toxic shock syndrome (STSS) and to life threatening (Nhan et al., 2012). *P. aeruginosa*, is responsible for potentially severe forms of dermatoses, such as *Ecthyma gangrenosum*, which marks immunosuppression or reveals septicemia, especially in children (Morand and Morand, 2017).

Antibiotic are usually used to treat infections caused by these pathogens microorganisms, but the increase of antibiotic resistance limits their efficacy. The unavailability of antibiotics because of high costs and inaccessibility to some of them is endemic to patients living in rural areas of sub-Saharan countries. In such a context, the search for new non-toxic molecules becomes imperative and natural substances from plants appear as viable alternative. Indeed, it was reported in some studies that some molecules from plants have properties to kill microbial agents (Górniak et al., 2019; Salas et al., 2015) or to inhibit antibiotic resistance mechanisms (Rao et al., 2018). These compounds act by several mechanisms such as the formation of complexes with proteins and polysaccharides possessing polyphenol function (Papuc et al., 2017); the disruption of microbial membranes by lipophilic flavonoids and terpenoids (Górniak et al., 2019), and the inhibition of microbial protein adhesion to host polysaccharide receptors (Lee et al., 2006). Thus, plant molecules because of their specificity play an important role in combating antibiotic resistance by inhibiting efflux pumps (Rao et al., 2018) and biofilm formation (Bhunu et al., 2017; Gupta et al., 2019). For example Fu et al. (2017) noted that *Herba patriniae*, a medicinal plant from the traditional Chinese pharmacopoeia, inhibited biofilm formation in *P. aeruginosa*. Elsewhere, Escobedo-Martínez et al. (2010) reported that pescaprine, a molecule isolated from *Ipomoea pes-capraea* had an inhibitory effect on Methicillin-resistant *S. aureus* (MRSA) efflux pumps, thus, increasing the norfloxacin effect. Some secondary plant metabolites also showed their synergistic role with other antibiotics. This was the case with oleanolic and ursolic acids, two pentacyclic triterpenes that increased the activity of Beta-lactam

antibiotics against *S. aureus*, *S. epidermidis* and *Listeria monocytogenes* strains (Kurek et al., 2012).

In Burkina Faso, the panorama of bacterial skin infections varies according to the region and is considered frequent (Andonaba et al., 2010; Korsaga et al., 2019). According to statistics from health Ministry skin diseases were the fifth reason for outpatient consultations in primary health Centers, in 2018. In Burkina Faso, *Cassia sieberiana*, *Cymbopogon schoenanthus*, *Lansea acida*, *Lawsonia inermis*, *Opilia celtidifolia*, *Piliostigma reticulatum*, *Pupalia lappacea*, *Sanseveria senegambica*, *Wissadula amplissima*, are frequently used by traditional healers to treat several infections including skin infections. Recipes developed are based on one or several plants (Guigma et al., 2012; Lengani et al., 2010; Zerbo et al., 2011). Decoction, trituration and aqueous maceration, infusion or carbonization is the main methods of preparing herbal concoctions. These herbal concoctions are administered through oral route and external applications. Regarding the widespread use of the nine aforementioned plant species in Burkina Faso, this study evaluated their *in vitro* antimicrobial activity against seven microbial strains causing skin infections.

MATERIALS AND METHODS

Microbial strains

Microorganisms involved in skin infections were selected and classified into group of Gram-positive cocci: *S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 and *S. agalactiae* ATCC 13813; Gram-negative bacilli: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27653 and fungal strains: *Candida albicans* ATCC 90028 and *Candida tropicalis* ATCC 750.

Plant materials

Plant materials were harvested from Gonsé forest and park Bangreweogo in June and October 2016. They were labelled and authenticated by botanists from "Centre National de la Recherche Scientifique et Technologique (CNRST)" and allocated particular voucher numbers. *Opilia celtidifolia* Guill. et Perr.: 8730; *Cassia sieberiana* (DC): 8731; *Lansea acida* A. Rich.: 8732; *Piliostigma reticulatum* (DC): 8733; *Lawsonia inermis* L: 8734; *Cymbopogon schoenanthus* (L.) Spreng. 8735; *Pupalia lappacea* (L): 8736; *W. amplissima* (L) Fries: 8737. Leaves, stem bark, and root (Table 1) were collected from each plant species when possible, then, thoroughly washed with clean water and dried in the shade at room temperature (25°C) until drying (7 to 14 days). The clean, dried plant materials were then pulverized into fine powder by using an electric grinder.

Preparation of the extracts

The extraction was performed according to the solid-liquid extraction method described by Bruneton Jean (1999) using solvents of increasing polarity (Bruneton, 1999) with slight modifications.

Decoction: 50 g of each plant material powder in 500 ml of distilled

Table 1. Plant species and material used.

Plant species	Family	Local name (mooré)	Parts used
<i>Opilia celtidifolia</i>	<i>Opiliaceae</i>	Wagsalga	Leaves Stem bark Root bark
<i>Cassia sieberiana</i>	<i>Caesalpiniaceae</i>	Kumbr-saka	Leaves Stem bark Root bark
<i>Lanea acida</i>	<i>Anacardiaceae</i>	Sabtulga	Leaves Stem bark Root bark
<i>Piliostigma reticulatum</i>	<i>Caesalpiniaceae</i>	Bagendé	Leaves Stem bark Root bark
<i>Lawsonia inermis</i>	<i>Lythraceae</i>	Djabi	Leaves
<i>Sanseveria senegambica</i>	<i>Asparagaceae</i>	Koantoabga	Root bark
<i>Cymbopogon schoenanthus</i>	<i>Poaceae</i>	Sompiiga	Leaves
<i>Wissadula amplissima</i>	<i>malvaceae</i>	Gomtilaogo	Leaves
<i>Pupalia lappacea</i>	<i>Amaranthaceae</i>	Voêns-tabdo	Leaves

water were boiled under reflux for 15 min. The cooled was filtered on a fine-mesh nylon tissue and then centrifuged at 2000 rpm for 10 min. The supernatant obtained was concentrated at 50°C in a ventilated oven. The concentrated extract was frozen at -24°C, and then freeze-dried. The dry extract was weighed and stored in the freezer waiting for biological tests.

Organic extraction: 50 to 100 g of plant powder was macerated for 24 h with 250 to 500 ml of ethanol (80 and 96%) or hexane. The extract was filtered on Whatman filter paper No5 and concentrated under reduced pressure with a rotavapor (BÜCHI R11, Germany). The concentrated extracts were then put in an oven at 40°C to remove the remaining solvent; the dried residues were weighed and also stored in the freezer for later use.

Preparation of the discs with the extracts, antibiotics and controls

The organic and aqueous extracts were each dissolved in dimethyl sulfoxide (DMSO) for analysis $\geq 99.8\%$ mixed with distilled water (1/5) to give an initial concentration of 300 mg/ml. This stock solution was then diluted to give final concentrations of 200 and 100 mg/ml. Erythromycin (15 μg /ml), Ciprofloxacin (5 μg /ml) and Nystatin (100 IU) were used as positive controls. The mixture of DMSO and water (1/5) was used as negative control. Sterile discs (6 mm diameter) were impregnated with plant extracts (300, 200 and 100 mg/ml) and / or positive and negative controls under a microbiological safety cabinet. They were placed in sterile Petri dishes and then dried in the oven at 37°C for 24 h.

In vitro testing of the antimicrobial activity

Preparation of the bacterial inoculum

A cryo-capsule containing the reference strain previously placed in an oven at 37°C for 10 to 15 min is dissolved in 1 ml of sterile

physiological water; 10 μl of the inoculum obtained were cultivated on an appropriate agar according to the growth conditions of microbial strains. The agar media were incubated for 24 h at 37°C for bacteria and 48 h at 37°C for fungi under strain-specific conditions. The morphological, cultural, biochemical and antigenic characteristics of microbial strains were carried out using standard and selective culture media as well as API kit. Colonies (1 to 2 wells) from the preculture were then introduced into a tube containing 5 ml of physiological saline water (0.9% NaCl). The turbidity was adjusted to the McFarland scale ($\sim 10^8$ CFU/ml) using a densitometer. The inoculum was reduced to 10^7 CFU/ml by dilution (1/10).

Agar disk-diffusion method

The antimicrobial activity testing was carried out using the disk-diffusion method of Kirby Bauer (SFM, 2011). Petri dishes containing Agar were inoculated with each inoculum previously prepared ($\sim 10^7$ CFU/ml) over their entire surface. Then, the dried discs containing the extracts (300, 200 and 100 mg/ml) as well as the control discs (negative and positive) were delicately placed on the agar surface of each petri dish. Petri dishes were incubated 24 h under growth conditions required for each microorganism. Zones of inhibition were measured (Figure 1). The test was validated if the negative control did not inhibit bacterial growth, and if the positive control had an inhibition diameter within the range given by the Clinical and Laboratory Standards Institute (CLSI).

Microdilution method

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of extracts (inhibition diameter ≥ 10 mm) were determined by the microdilution method (CLSI, 2012). In a 96-well microplate, successive dilutions of reason 2, were made from wells to wells (wells 1 to 6), leading to final concentrations varying between 150 and 0.0183 mg / ml. The wells 7 and 8 were used for positive and negative controls. The bacterial

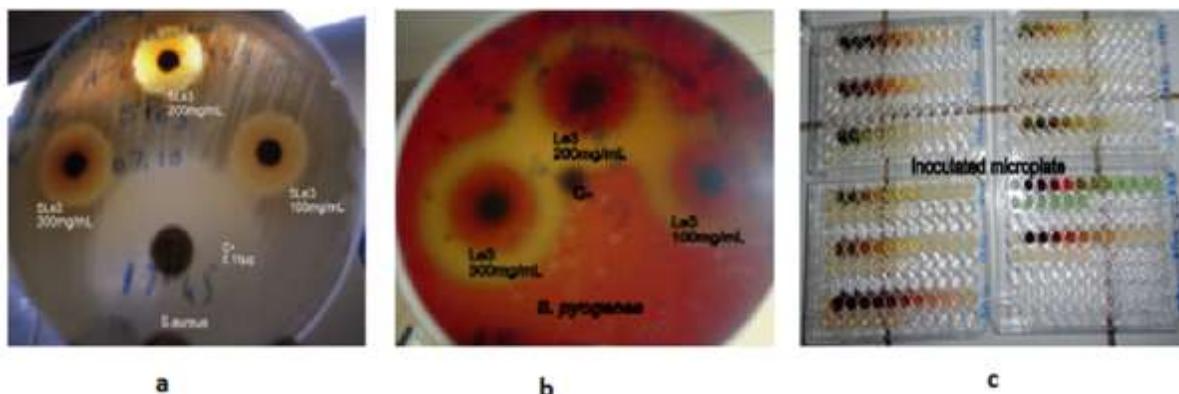


Figure 1. Determination of inhibition zones of the ethanolic extract (80%) of the leaves of *Lawsonia inermis* on *Staphylococcus aureus* (a) and *Streptococcus pyogenes* (b); inoculated microplates (c).

inoculum in a broth (Mueller Hinton, Heart-Brain or Sabouraud) at 10^6 CFU/ml was added to each well; the microplates were incubated at 37°C for 18 h. The MIC was the lowest concentration of extract that completely inhibits the growth of the bacteria in wells, estimated visually compared to controls. Plates containing MH, Sabouraud or chocolate + polyvitex agar, inoculated with $5\ \mu\text{l}$ of the inoculum from wells which did not show visible growth when determining the MIC were sub-cultivated; CMB was the lowest concentration of the extract with no colony growth. The MBC/MIC ratio allows to specify the action mode of a substance. If the MBC/MIC ratio is ≤ 2 , the substance is bactericidal. On the other hand, if it is ≥ 2 , the substance is bacteriostatic (Fauchere, 2002).

Statistical analysis

Data were analyzed with Excel 2010. Results are expressed as mean (3 tests) plus standard deviation. The Student t-test was applied for means comparison with the significance level set at 5%.

RESULTS AND DISCUSSION

Antibacterial and antifungal activity of the extracts

In total, 68 extracts among which leaf extracts (32), root extracts (20) and stem bark extracts (16) were tested against five strains of bacteria and two strains of fungi, American Type Culture Collection (ATCC). At 300 mg/ml, 28 extracts (41%) from eight plant species had at least a diameter of inhibition ≥ 10 mm) on four of the bacteria strains; at 200 mg/ml, 18 (27%) extracts had this diameter, and at 100 mg /ml, only 7 (10%) extracts. The active extracts showed a dose-dependent inhibition on bacteria and fungi.

The inhibitory activity was variable regarding the plant parts. Among extracts with an inhibition zone ≥ 10 mm, leaf extracts were the most represented, (14) followed by stem bark extracts (09); while only 5 extracts from root bark were recorded.

According to Habbal et al. (2011), the variable antimicrobial activity displayed by the extracts may

depend on the type of extraction solvent, the part of the plant, the concentration and the microorganism strain (Habbal et al., 2011). It was also shown that chlorophyll, which is one of the constituents of fresh leaves possess antimicrobial activity (Maekawa et al., 2007). However, the high proportion of leaves among the collected samples (32 samples out of 68), could justify these results.

Extracts with medium to high polarity (inhibition diameter ≥ 10 mm) were the most active. There were 18 ethanol extracts, 9 decoctions and one non-polar extract (hexan extract).

The ethanolic and aqueous extracts of the leaves of *L. inermis* displayed the highest effect on *S. pyogenes* (20 mm) and *S. aureus* (19.67 mm), at 100-300 mg/ml. Only, the hexane extract of *C. schoenanthus* (Le1) (Table 2) gave a visible inhibition zone (9-10 mm) on *S. agalactiae* and (10-12 mm) on *C. tropicalis* at 100-300 mg/ml. This reflected the ability of polar solvents to retain the active antimicrobial ingredients; which in polar extracts are mainly tannins, saponins, flavonoids and alkaloids (Abdulrazak et al., 2015; Górnaiak et al., 2019; N'Guessan et al., 2015; Owusu and Ofori-Amoah, 2017). On the other hand, the low activity of hexane extracts could be explained by the absence or the low concentration of potent antimicrobial compounds in the solvent which is known to better precipitates essential oils. This could justify the results observed with the leaf extracts of *C. schoenanthus*. However, Hashim et al. (2016) have demonstrated the efficacy of *C. schoenanthus* essential oils to inhibit several bacterial strains. No extract significantly inhibited the growth of *E. coli*.

However, only the aqueous extract of *L. inermis* inhibited the growth of *P. aeruginosa* with an inhibition diameter of 10.67 mm at 300 mg/ml (Table 2). Twenty (29%) extracts inhibited the growth of *S. aureus* with an inhibition diameter ranging from 10 to 19.67 mm, while nine others (18%) diversely inhibited the growth of *S. pyogenes*.

Table 2. Inhibition zones (IZ) of the plants extracts on the bacterial strains (IZ \geq 10 mm).

Plants	Extracts	Inhibition zone diameter, Mean \pm SD (mm)		
		100 mg/ml	200 mg/ml	300 mg/ml
<i>Staphylococcus aureus</i>				
<i>Opilia celtidifolia</i>	Le4	9.67 \pm 0,4	10 \pm 0	10.33 \pm 0,4
	SB4	12.67 \pm 0.4	13 \pm 0	13.67 \pm 0.44
<i>Cassia sieberiana</i>	Le2	-	-	10.67 \pm 0.4
	Le4	-	10.67 \pm 0,8	12.17 \pm 0,2
	SB2	-	10 \pm 0	11.33 \pm 0.4
	SB3	-	-	10.67 \pm 0.4
	SB4	-	-	10 \pm 0.6
	RB2	-	-	11 \pm 0.6
<i>Lansea acida</i>	SB2	-	10 \pm 0.6	10.5 \pm 0.6
	SB4	-	-	10.33 \pm 0.4
	RB3	-	-	10.67 \pm 0.4
<i>Piliostigma reticulatum</i>	Le2	-	-	11.67 \pm 0,6
	Le3	-	-	11.67 \pm 0.6
	SB2	-	10 \pm 0.6	11.33 \pm 0.8
	SB3	-	10.67 \pm 0.8	11.83 \pm 0.5
<i>Lawsonia inermis</i>	Le2	10 \pm 0	11,33 \pm 0,4	13.67 \pm 0.8
	Le3	13 \pm 0	15 \pm 0.6	17 \pm 0.6
	Le4	15 \pm 0,6	17 \pm 0.4	19.67 \pm 0.8
<i>Sanseveria senegambica</i>	RB4	9.33 \pm 0.4	10.33 \pm 0.4	10.33 \pm 0.4
<i>Pupalia lappacea</i>	Le4	11 \pm 0	12 \pm 0.6	13.33 \pm 0.8
<i>Streptococcus pyogenes</i>				
<i>Opilia Celtidifolia</i>	RB2	10,33 \pm 3.2	12.67 \pm 4.7	17.67 \pm 4.04
	RB3	-	-	12 \pm 0
<i>Cassia sieberiana</i>	Le4	0	9.33 \pm 0.6	11 \pm 0
	SB2	-	-	10 \pm 1
<i>Lawsonia inermis</i>	Le2	10 \pm 0	11 \pm 0	13 \pm 0
	Le3	16 \pm 3.6	18 \pm 3.6	20 \pm 4
<i>Cymbopogon schoenanthus</i>	Le4	-	10 \pm 0	11.33 \pm 0.6
<i>Pupalia lappacea</i>	Le3	-	13.33 \pm 4.1	17.33 \pm 5.8
	Le2	-	-	12 \pm 1
<i>Streptococcus agalactiae</i>				
<i>Opillia celtidifolia</i>	RB2			10.33 \pm 0.6
	RB3	-	13 \pm 2.6	13 \pm 2.6
<i>Lawsonia inermis</i>	Le2	-	11 \pm 4.3	12.33 \pm 5.9
	Le3	10.67 \pm 2.3	12 \pm 4	13.33 \pm 3
<i>Cymbopogon schoenanthus</i>	Le1	-	10 \pm 0	11 \pm 0
<i>Pseudomonas aeruginosa</i>				
<i>Lawsonia inermis</i>	Le4	-	10.67 \pm 0.6	10.67 \pm 0.6
<i>Candida albicans</i>				
<i>Opillia celtidifolia</i>	Le4	19 \pm 1	20.67 \pm 0.6	21.67 \pm 1.5
	SB4	21 \pm 1	22.67 \pm 1.1	24.33 \pm 0.6

Table 2. Cont.

	<i>Candida tropicalis</i>			
<i>Lansea acida</i>	Le2	-	-	12.33±0
	Le4	-	10	11±0
<i>Lawsonia inermis</i>	Le2	-	-	10.33±0.6
	Le3	-	-	10.67±0.6
<i>Cymbopogon schoenanthus</i>	Le ₁	10.67±0.6	11.67±0.6	12±0

Leaves extracts= Le; Stem Bark = SB; Root bark=RB; n-hexane: =1; ethanol 96%: =2 ethanol 80%: =3; water: =4; (-) =no active.

The results on Gram-negative bacilli (GNBs) contrasted with those of other studies with the same plants species; (Chowdhury et al., 2014; Habbal et al., 2011; Hoekou et al., 2012). Biotic and abiotic factors, harvesting period, parts of the plant used and extraction method may explain these differences (Mukherjee and Houghton 2009). The activity displayed by *L. inermis* on *P. aeruginosa* may be attributed to free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive (Al-Rubiay et al., 2008). In addition, to providing a source of stable free radicals, compounds such as quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function (Habbal et al., 2011).

Most of the extracts fell to inhibited Gram-negative bacilli; this result was not surprising. Indeed, it was reported that Gram-negative bacilli (GNBs) have an intrinsic resistance to biocidal agents because of the nature of their outer membranes (Dzidic et al., 2008). The same reason may explain the high susceptibility of GPCs found in the study. Gram-positive bacteria walls are made exclusively of peptidoglycans, which seems to be more favorable to the penetration of phytomolecules in bacteria (Dzidic et al., 2008).

7 (10%) extracts were able to inhibit the growth of fungi among which the aqueous extracts of the leaves and stem barks of *O. celtidifolia* (1Le4 and 1SB4) had almost similar antifungal activity as the control (nystatin) on *C. albicans* (21,67 and 24,33 mm). The antifungal activity of these extracts may be due to the different chemical compounds (phenolics, tannins, phlobatannins, alkaloids, triterpenoid saponins, anthraquinones and flavanoid) present in *O. celtidifolia* (Mazadu et al., 2018). It has been reported that two flavonoid classes (flavans and flavanols) owing to their special structure formed by hydroxyl groups in position 2, 4, 5 and 7 on the aromatic core possess significant antimicrobial power (Bouterfas et al., 2016). However, the optimal efficacy of a medicinal plant may not be due to one main active ingredient, but to the combined action of different compounds originally in the plant (Essawi et Srour, 2000). Therefore, there is no explanation for the difference in inhibition depending on the fungal species observed.

Fifteen (22%) of the extracts had an inhibition zone less

than 10 mm ($d < 10$ mm) and were considered as inactive to weakly active. They included all leaf extracts of *W. amplissima* (4) and 11 extracts from the other 8 plants. It is unusual to notice significant differences in data for the same plant species because important variations of activity can be observed with extracts of plants treated by solvents presenting very polar characters. Thus, between an aqueous extract and an alcoholic extract of low titer, important pharmacological differences appear and can particularly be concretized by a lack of activity (Bouharb et al., 2014).

These variations might be due to many factors, including the method of extraction, climatic, seasonal and geographical conditions, and harvest time as previously notified. There were no similar studies to compare the non-activity of *W. amplissima* extracts. However, the period (June and October) of samples collection that corresponds to the middle and end of the winter season could be a limiting factor because the plants are still full of large quantity of water which may dilute the active ingredients.

All the strains were sensitive to reference antibiotics (ciprofloxacin, erythromycin and antifungal (Nystatin). Their activity was clearly superior to that of all the plant extracts (21.5 to 34 mm for bacteria and 23.51 to 23.76 mm for fungi). The difference of activity between pure chemical compounds and plant extracts has been argued and the most plausible explanation was the low concentration of active ingredients and the presence of both synergistic and antagonistic chemical constituents in the same extracts.

Minimal inhibitory concentration and minimal bactericidal concentration of the plant extracts

The MIC and MBC values varied depending on the types of strains. Extracts with the best MIC (< 1 mg/ml) and MBC are presented in Table 4, showing that 11 extracts from 7 of the plants had a MIC < 1 mg/ml, among which 8 had a bactericidal effect (MBC/MIC = 2) and the 3 others a bacteriostatic effect (MBC/MIC ≥ 4). The susceptibility of Gram-positive cocci (GPC) to the extracts was confirmed with the MIC. Ethanol extracts (96 and 80%) of *O. celtidifolia* root barks were the most effective (MIC between 0.03 and 0.24 mg/ml) on Gram-positive cocci, *S.*

Table 3. Inhibitory zones with the standard antibiotics and antifungals.

Antibiotic	Microbial strains	Inhibition zone diameter: Mean \pm SD (mm)		
		Essay 1	Essay 2	Essay 3
Ciprofloxacin (5 μ g)	<i>Pseudomonas aeruginosa</i>	28.85 \pm 1.45	28.51 \pm 1.07	28.86 \pm 0.96
	<i>Escherichia coli</i>	31.91 \pm 0.9	32.04 \pm 0.5	31.69 \pm 0.7
Erythromycin (15 μ g)	<i>Staphylococcus aureus</i>	27.5 \pm 0.7	27.8 \pm 0.49	27.91 \pm 0.5
	<i>Streptococcus pyogenes</i>	34 \pm 0	33.91 \pm 0.28	33.83 \pm 0.40
	<i>Streptococcus agalactiae</i>	31.63 \pm 0.48	31.55 \pm 0.5	31.72 \pm 0.64
Nystatin (100 UI)	<i>Candida albicans</i>	23.66 \pm 0.8	23.76 \pm 0.42	23.70 \pm 0.45
	<i>Candida tropicalis</i>	23.54 \pm 0.78	23.75 \pm 0.43	23.73 \pm 0.58

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the plant extracts.

Plants	Extract	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Interpretation
<i>Staphylococcus aureus</i>					
<i>Lawsonia inermis</i>	Le2	0.41 \pm 0.29	1.56 \pm 0.67	3.80	Bactericidal
<i>Lannea acida</i>	SB2	0.98 \pm 0.33	4.69 \pm 0	4.78	Bacteriostatic
<i>Streptococcus pyogenes</i>					
<i>Lawsonia inermis</i>	Le2	0.29 \pm 0	1.17 \pm 0	4	Bactericidal
	Le3	0.58 \pm 0	1.17 \pm 0	2	Bactericidal
<i>Cassia sieberiana</i>	SB2	0.49 \pm 0.17	3.91 \pm 1.35	7.98	Bacteriostatic
<i>Opilia celtidifolia</i>	RB2	0.06 \pm 0.02	0.07 \pm 0	1.17	Bactericidal
	RB3	0.03 \pm 0.01	0.03 \pm 0.01	1	Bactericidal
<i>Streptococcus agalactiae</i>					
<i>Lawsonia inermis</i>	Le2	0.48 \pm 0.17	0.97 \pm 0.33	2	Bactericidal
	Le3	0.78 \pm 0.33	3.91 \pm 1.35	5.01	Bacteriostatic
<i>Opilia celtidifolia</i>	RB2	0.07 \pm 0	0.15 \pm 0	2	Bactericidal
	RB3	0.24 \pm 0.08	0.58 \pm 0	2.42	Bactericidal

Leaves extracts= Le; Stem Bark = SB; Root bark=RB; n-hexane =1; ethanol 96% =2, ethanol 80% =3; water =4.

pyogenes and *S. agalactiae* (Table 3). Ethanolic extracts (96 and 80%) of the leaves of *L. inermis* exercised moderate inhibitory activity (MIC between 0.29 and 0.78 mg/ml) on susceptible strains. Among all the extracts, only, the 96% ethanolic extract of the leaves of *L. inermis* had a bactericidal effect on *S. aureus*.

The MICs of ethanol leaf extracts of *L. inermis* for susceptible strains were higher than what reported by previous studies (Al-Rubiay et al., 2008). This discrepancy may be due to many of the factors previously mentioned.

The efficacy of *O. celtidifolia* extracts could be explained by its richness in various antimicrobial compounds. Previous phytochemical screenings on *O. celtidifolia* showed several phytochemical compounds such as flavonoids (Mazadu et al., 2018), tannins (Mazadu

et al., 2018), alkaloids (Mazadu et al., 2018; Owolabi et al., 2017), terpenoids (Owolabi et al., 2017), coumarins (Togola et al., 2015), polysaccharides of pectin type II (Inngjerdingen et al., 2013), and unsaturated fatty acids (ximenynic acid) (Traore, 2008). These phytochemicals may be responsible of the antibacterial (Górniak et al., 2019; Lopes et al., 2017; Papuc et al., 2017) and antifungal (Merghache et al., 2012) activities of the plant extracts. Furthermore, Korotimi (2010) reported the wound-healing effect of the leaves extract of *O. celtidifolia* in Mali.

Conclusion

The results obtained during this study confirmed the

antimicrobial properties of some of the selected plants and could justify their use in traditional medicine against skins infectious of bacterial and fungal causes. The extracts from *Opilia celtidifolia* and *Lawsonia inermis* with the most promising antimicrobial activity will be further investigated for their development into improved traditional phytomedicines.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Abdulrazak N, Asiya UI, Usman NS, Unata IM, Farida A (2015). Antiplasmodial activity of ethanolic extract of root and stem bark of *Cassia sieberiana* DC on mice. *Journal of Intercultural Ethnopharmacology* 4(2):96-101. <https://doi.org/10.5455/jice.20141231014333>
- Al-Rubiay KK, Jaber NN, Alrubaiy LK (2008). Antimicrobial efficacy of henna extracts. *Oman Medical Journal* 23(4):253-256. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3273913&tool=pmcentrez&rendertype=abstract>
- Andonaba J, Barro-Traoré F, Diallo B, Sakana L, Niamba P, Traoré A (2010). Aspects épidémiologiques des affections dermatologiques au Centre Hospitalier Universitaire Sourou SANOU de Bobo-Dioulasso. *Annales Africaines de Médecine* 4(1):668-677.
- Bhunu B, Mautsa R, Mukanganyama S (2017). Inhibition of biofilm formation in *Mycobacterium smegmatis* by *Parinari curatellifolia* leaf extracts. *BMC Complementary and Alternative Medicine* 17(1):110. <https://doi.org/10.1186/s12906-017-1801-5>
- Bouharb H, Badaoui K, Zair T, Amri J, Chakir S, Alaoui T (2014). Sélection de quelques plantes médicinales du Zerhoun (Maroc centrale) pour l'activité antibactérienne contre *Pseudomonas aeruginosa*. *Journal of Applied Biosciences* 78:6685-6693 ISSN 1997-5902
- Bouterfas K, Mehdadi Z, Aouad L, Elaoufi MM, Khaled MB, Latreche A, Benchiha W (2016). La localité d'échantillonnage influence-t-elle l'activité antifongique des flavonoïdes de *Marrubium vulgare* vis-à-vis de *Aspergillus niger* et *Candida albicans*? *Journal de Mycologie Médicale* 26(3):201-211. <https://doi.org/10.1016/j.mycmed.2016.02.019>
- Bruneton J (1999). *Pharmacognosie, phytochimie, plantes médicinales* (Lavoisier (ed.); 4e ed.). Tec et Doc édition1.
- Chowdhury MMH, Kubra K, Ahmed SR (2014). Antimicrobial, phytochemical and toxicological evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. *Research Journal of Medicinal Plant* 8(4):187-195. <https://doi.org/10.3923/rjmp.2014.187.195>
- CLSI (Clinical and Laboratory Standards Institute). (2012). *Performance Standards for Antimicrobial Disk Susceptibility Tests*; Approved Standard - Eleventh Edition (R. (Cockerill) Franklin, A. (FIDSA) Mathew, A. Jeff, N. (FIDSA) Michael, G. M. (Eliopoulos), J. F. (MPH) Mary, A. H. A. Janet, B. P. (ABMM) Jean, P. (FRCPATH) Mair, & M. T. (ASCP) Maria (eds.); 11th ed., Vol. 32, Issue 1). ISSN 2162-2914 (Electronic). <https://doi.org/www.clsi.org/standard@clsi.org>
- Dione H, Bammo M, Lawson ATD, Seck F, Dioussé P, Guèye N, Faye FA, Touré PS (2018). Les urgences dermatologiques à l'hôpital régional de Thiès/Sénégal: une série de 240 cas. *Revue Africaine de Médecine Interne* 5(1):11-14.
- Dzidic S, Suskovic J, Kos B (2008). Antibiotic Resistance Mechanisms in Bacteria: Biochemical and Genetic Aspects. *Food Technology Biotechnology* 46(1):11-21.
- Escobedo-Martínez C, Cruz-Morales S, Fragozo-Serrano M, Mukhlesur RM, Gibbons S, Pereda-Miranda R (2010). Characterization of a xylose containing oligosaccharide, an inhibitor of multidrug resistance in *Staphylococcus aureus*, from *Ipomoea pes-caprae*. *Phytochemistry* 71(14-15):1796-1801. <https://doi.org/10.1016/j.phytochem.2010.06.018>
- Essawi T et Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology* 70(3):343-349. [https://doi.org/10.1016/S0378-8741\(99\)00187-7](https://doi.org/10.1016/S0378-8741(99)00187-7)
- Fauchere JL (2002). *Bactériologie Générale et Médicale*, (Editions Ellipses).
- Fofana Y, Traore B, Dicko A, Faye O, Berthe S, Cisse L, Keita A, Tall K, Kone MB, Keita S (2016). Profil épidémiologique des dermatoses chez les enfants vus en consultation dermatologique dans le service de dermatologie du centre national d'appui à la lutte contre la maladie à Bamako (Mali). *Pan African Medical Journal* 25:1-6. <https://doi.org/10.11604/pamj.2016.25.238.10564>
- Fu B, Wu Q, Dang M, Bai D, Guo Q, Duan K, Shen L (2017). Inhibition of *Pseudomonas aeruginosa* Biofilm Formation by Traditional Chinese Medicinal Herb *Herba patriniae*. *BioMed Research International*, pp.1-10. <https://doi.org/10.1155/2017/9584703>
- Górniak I, Bartoszewski R, Króliczewski J (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews* 18(1): 241-272. <https://doi.org/10.1007/s11101-018-9591-z>
- Guigma Y, Zerbo P, Millogo-Rasolodimby J (2012). Utilisation des espèces spontanées dans trois villages contigus du Sud du Burkina Faso. *Tropicicultura* 30(4):230-235.
- Gupta K, Singh SP, Manhar AK, Saikia D, Namsa ND, Konwar BK, Mandal M (2019). Inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilm and Virulence by Active Fraction of *Syzygium cumini* (L.) Skeels Leaf Extract: In-Vitro and In Silico Studies. *Indian Journal of Microbiology* 59(1):13-21. <https://doi.org/10.1007/s12088-018-0770-9>
- Habbal O, Hasson SS, El-Hag AH, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, Al-Balushi MS, Al-Jabri AA (2011). Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Biomedicine* 1(3):173-176. [https://doi.org/10.1016/S2221-1691\(11\)60021-X](https://doi.org/10.1016/S2221-1691(11)60021-X)
- Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, Marks R, Naldi L, Weinstock MA, Wulf SK, Michaud C, Murray JIC, Naghavi M (2014). The global burden of skin disease in 2010: An analysis of the prevalence and impact of skin conditions. *Journal of Investigative Dermatology* 134(6):1527-1534. <https://doi.org/10.1038/jid.2013.446>
- Hoekou YP, Batawila K, Gbogbo KA, Karou DS, Ameyapoh Y (2012). Evaluation des propriétés antimicrobiennes de quatre plantes de la flore togolaise utilisées en médecine traditionnelle dans le traitement des diarrhées infantiles. *International Journal of Biological and Chemical Sciences* 6(12):3089-3097. <https://doi.org/dx.doi.org/10.4314/ijbcs.v6i6.10>
- Inngjerdigen KT, Langerud BK, Rasmussen H, Olsen TK, Austerheim I, Grønhaug TE, Aaberge IS, Diallo D, Paulsen BS, Michaelsen TE (2013). Pectic Polysaccharides Isolated from Malian Medicinal Plants Protect against *Streptococcus pneumoniae* in a Mouse Pneumococcal Infection Model. *Scandinavian Journal of Immunology* 77(5):372-388. <https://doi.org/10.1111/sji.12047>
- Korotimi DAK (2010). *Propriété cicatrisante des feuilles de Opilia celtidifolia* (Guill. et Perr.) Endl. ex Walp. Doctorate thesis in Pharmacy, University of Bamako, pp. 1-109.
- Korsaga/Somé N, Zoungrana/Ouédrogo A, Konaté I (2019). Skin

- disorders in preschool environment in the city of Ouagadougou (Burkina Faso): Epidemiological, clinical and therapeutic aspects. *Our Dermatology Online* 10(e):e31.1-e31.8. <https://doi.org/10.7241/ourd.2019e.31>
- Kurek A, Nadkowska P, Pliszka S, Wolka KI (2012). Modulation of antibiotic resistance in bacterial pathogens by oleanolic acid and ursolic acid. *Phytomedicine* 19(6):515-519. <https://doi.org/10.1016/j.phymed.2011.12.009>
- Lee JH, Jin SS, Jung SL, Jin KK, In SY, Chung MS, Kyung HK (2006). Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*). *Journal of Agricultural and Food Chemistry* 54(23):8717-8723. <https://doi.org/10.1021/jf061603i>
- Lengani A, Lompo LF, Guissou IP, Nikiema JB (2010). Médecine traditionnelle et maladies des reins au Burkina Faso. *Néphrologie et Thérapeutique* 6(1):35-39. <https://doi.org/10.1016/j.nephro.2009.07.011>
- Lopes G, Pinto E, Salgueiro L (2017). Natural Products: An Alternative to Conventional Therapy for Dermatophytosis? *Mycopathologia* 182(1-2):143-167. <https://doi.org/10.1007/s11046-016-0081-9>
- Maekawa LE, Lamping R, Maekawa MY, Renata M, Nassri G (2007). Antimicrobial activity of chlorophyll-based solution on *Candida albicans* and *Enterococcus faecalis*. In *Vitro*, November 2007.
- Marks M, Sammut T, Cabral MG, Teixeira E, Nakutum J, Goncalves A, Rodrigues A, Ca J, Id UDA, Achan J, Logan J, Bailey R, Mabey D, Last A, Walker SL (2019). The prevalence of scabies, pyoderma and other communicable dermatoses in the Bijagos Archipelago, Guinea-Bissau. *PLOS Neglected Tropical Diseases* 12:1-9. <https://doi.org/https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007820>
- Mazadu EA, Misau MS, Gwallameji LB (2018). Phytochemical screening and antimicrobial activity of some medicinal trees grown in Bauchi state, north eastern, Nigeria. *Journal of Pharmacognosy and Phytochemistry* 7(2):3503-3507.
- Merghache D, Boucherit-Atmani Z, Boucherit K (2012). Evaluation of the antifungal activity of different extracts of Chinese cinnamon (*Cinnamomum cassia*). *Phytothérapie* 10(4):215-221. <https://doi.org/10.1007/s10298-012-0718-x>
- Morand A, Morand JJ (2017). *Pseudomonas aeruginosa* in dermatology. *Annales de Dermatologie et de Vénérologie* 10. <https://doi.org/10.1016/j.annder.2017.06.015>
- Mukherjee Pk, Houghton P. (2009). Evaluation of herbal medicinal products. *Pharmaceutical Press* 1:519.
- N'Guessan, BB, Dosso K, Gnangoran BN, Amoaeng P, Asiedu-Gyekye IJ, Yapo AP (2015). Antibacterial and antispasmodic activities of a dichloromethane fraction of an ethanol extract of stem bark of *Piliostigma reticulatum*. *Journal of Pharmacy and Bioallied Sciences* 7(2):128-135. <https://doi.org/10.4103/0975-7406.154439>
- Nhan TX, Gillet Y, Vandenesch F (2012). Diagnostic et traitements des infections toxiques à *Staphylococcus aureus*. *Journal Des Anti-Infectieux* 14(3):117-126. <https://doi.org/10.1016/j.antinf.2012.07.001>
- Owolabi MS, Omowonuola AA, Lawal OA, Dosoky NS, Collins JT, Ogungbe IV, Setzer WN (2017). Phytochemical and bioactivity screening of six Nigerian medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 6(6):1430-1437.
- Owusu G, Ofori-Amoah J (2017). Anti-inflammatory and Analgesic Effects of an Aqueous Extract of *Lannea acida* Stem Bark. *British Journal of Pharmaceutical Research* 16(6):1-8. <https://doi.org/10.9734/BJPR/2017/33266>
- Papuc C, Goran GV, Predescu CN, Nicorescu V, Stefan G (2017). Plant Polyphenols as Antioxidant and Antibacterial Agents for Shelf-Life Extension of Meat and Meat Products: Classification, Structures, Sources, and Action Mechanisms. *Comprehensive Reviews in Food Science and Food Safety* 00, 1-26. <https://doi.org/10.1111/1541-4337.12298>
- Rao M, Padyana S, Dipin KM, Kumar S, Nayak BB, Varela MF (2018). Antimicrobial Compounds of Plant Origin as Efflux Pump Inhibitors: New Avenues for Controlling Multidrug Resistant Pathogens. *Journal of Antimicrobial Agents* 04(01):1-6. <https://doi.org/10.4172/2472-1212.1000159>
- Salas CE, Badillo-Corona JA, Ramirez-Sotelo G, Oliver-Salvador C (2015). Biologically active and antimicrobial peptides from plants. *BioMed Research International*, 2015. <https://doi.org/10.1155/2015/102129>
- SFM (Société Française de Microbiologie) (2011). Comité de l'Antibiogramme de la Société Française de Microbiologie: Recommandations 2011 (C. J. (CHU/Henri M. Soussy, R. Bonnet, J. D. Cavallo, H. Chardon, C. Chidiac, P. Courvalin, H. Dabernat, & Drugeon (eds.)).
- Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J (2012). *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews* 36(2):288-305. <https://doi.org/10.1111/j.1574-6976.2011.00278.x>
- Togola A, Karabinta K, Denou A, Haidara M, Sanogo R, Diallo D (2015). Effet protecteur des feuilles de *Opilia celtidifolia* contre l'ulcère induit par l'éthanol chez le rat. *International Journal of Biological and Chemical Sciences* 8(6):2416. <https://doi.org/10.4314/ijbcs.v8i6.5>
- Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews* 28(3):603-661. <https://doi.org/10.1128/CMR.00134-14>
- Traore M (2008). Phytochemical studies of plants used as antimalarials in Burkina Faso, PhD thesis, university of Copenhagen.
- Zerbo P, Millogo-rasolodimby J, Nacoulma-ouedraogo OG, Van Damme P (2011). Plantes médicinales et pratiques médicales au Burkina Faso : cas des Sanan. *Bois et Forêts Des Tropiques* 307(1):41-53.