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ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF WHOLE PLANT EXTRACTS OF *Setaria barbata* (*Poaceae*) AND OF LEAVES AND FRUITS OF *Pinus sylvestris* (*Pinaceae*)

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ABSTRACT

Exploration through plants used in phytotherapy is one of the requirements to the current struggle against infectious diseases with the increasing microbial resistance to conventional drugs. The present investigation aimed at assessing the antibacterial and antifungal potential of the fresh whole plant of *Setaria barbata* (*Poaceae*) and, of the dried leaves and fruits of *Pinus sylvestris* (*Pinaceae*) harvested in Cameroon. Subsequent to harvesting, cleaning and grinding, extraction was conducted by maceration in 70% ethanol and distilled water. The extracts were subjected to phytochemical screening, prior to investigation through the minimum inhibitory, bactericidal and fungicidal concentrations (MIC, MBC, MFC, respectively); and activity type. Test organisms comprised *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. hominis*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Candida albicans*, *Trichophyton rubrum*, *T. mentagrophytes*, *T. torsurans*, and *T. interdigitale*. The phytochemical screening revealed groups of secondary metabolites in

which the larger diversity was observed in the hydro-ethanol extracts. All extracts were active on subjected microorganisms. The MIC values globally varied from 0.195 mg/mL through 50 mg/mL. The MLCs varied from 25 mg/mL through 200 mg/mL in bacteria and 25 mg/mL through 400 mg/mL in fungi. Overall, extracts exhibited bacteriostatic and fungistatic activities. These results support the use of the leaves and the fruits of *P. sylvestris* and *S. barbata* for therapeutic purposes in case of infections; but additional investigations are necessary to address the sustainable use of the extracts in infection control.

KEYWORDS: *Setaria barbata*, *Pinus sylvestris*, medicine, antibacterial, antifungal potential.

INTRODUCTION

Investigation into plants used in phytotherapy is one of the most important challenges to meet for global health issues including infectious diseases (IDs). Infectious diseases basically refer to physiological disorders associated with microorganisms. They include bacteria, viruses, protozoa and microscopic fungi.[1] Despite tremendous advances in science and technology which enabled the discovery of several natural and synthetic drugs, IDs continue to cause heavy burdens in terms of morbidity and mortality; representing therefore, serious handicap to healthcare systems worldwide and especially in low- and middle-income countries.[2] According to current reports, they are responsible for half of all deaths recorded in tropical countries.[3] Also, about 90% of infections are caused by bacteria[4] with more and more frequent related threats. In line with these reports, at least 30 new IDs for which no effective treatment is known emerged since the 1970s.[5] The situation is becoming increasingly complex with the emergence of antimicrobial-resistant microorganisms and uncommon infections that undermine caretaking initiatives with available drug regiments.[6] In Cameroon and other developing countries, IDs represent a real cause of concern based on their number, their frequency and their severity.[7,8]

One of the critical steps towards controlling IDs for safer life is the use of effective new antimicrobial agents with variable spectra. Acknowledging that more than 80% of the most exposed world populations rely on natural resources for health services, the WHO and other influential health system partners encouraged research through natural solutions that could serve as alternative to current conventional arsenals for which availability and affordability are not warrantee for most needy groups. Based on their potentials, therapy with plant derivatives emerged as a promising option. Researches for plant-derived anti-infective agents

have therefore become priorities duties for many research groups throughout the world. Consequently, large numbers of studies have been initiated and conducted on the antibacterial and antifungal potentials of *Setaria barbata* (*Poaceae*) and *Pinus sylvestris* (*Pinaeae*).[9] In addition to gastrointestinal disorders, the leaves and buds of *Pinus sylvestris* (*P. sylvestris*) are used for microbial-related conditions of the respiratory tract (cough, cold sore, pharyngitis, rhinitis, bronchitis), and skin infections.[10,11] The present study aimed at providing additional pieces of information on the antimicrobial potential of these plants through *in vitro* investigation on their extracts. More specifically, the anti-bacterial and anti-fungal minimal inhibitory and lethal concentrations (MIC and MLC, respectively) of the hydro-ethanol and aqueous extracts of the leaves and fruits of *P. sylvestris;* and those of the hydro-ethanol extract of the whole *Setaria barbata* (*S. barbata*) grown in Cameroon were assessed on selected bacterial and fungi isolates. Prior to this antimicrobial potential, the phytochemical screening was conducted for a few key plant phytochemical groups. Major findings revealed at varied levels, the antimicrobial potentials of these extracts. They also indicated that their potential was not dependent on the richness in metabolites, but most likely (though yet to be supported by additional studies) on the phytochemical's. Overall, these pieces of information will help in the short run to update the potential of these plants. In the intermediate and long run, they will help refine their use for more effectiveness at the primary healthcare level and will, at least partially substitute the use of conventional drugs.

MATERIAL AND METHODS

Type and population of the study

The present cross-sectional descriptive survey was conducted with the leaves and fruits of *Pinus sylvestris* (*P. sylvestris*) and the whole fresh *Setaria barbata* (*S. barbata*) plant.

Plant collection and first treatment

Leaves and fruits of *P. sylvestris* were collected in January 2019, in Banekane (Ndé Division, West-Cameroon). At the identification, it was noticed that it did not have any representing specimen at the National Herbarium of Cameroon (NHC). The specimens were cleared of waste and dried at room temperature (≈ 22°C) in a dark container for twelve days. The resulting raw material was separately crushed into powders with an electric grinder and the powders stored in dark containers between 4-6°C until use.

Whole fresh *S. barbata* plants were collected in Yaoundé (Centre-Cameroon) in March 2019 and identified at the National Herbarium under reference number 36829/NHC. Once

collected, it was cleaned and crushed into a paste in a mortar. This final product was preserved between 4-6°C until used.

Hydro-alcoholic and aqueous extraction

Hydro-alcoholic extraction

To have the hydro-alcoholic extract used in the present survey, 1500 mL of 70% ethanol was used to macerate 500 grams of the dry powders from the leaves and fruits of *P. sylvestris.* The same protocol was used with the whole crushed fresh plant of *S. barbata*. Maceration was conducted for 48 hours with renewed solvent after 24 hours for both raw products. The macerate was thereafter concentrated overnight in a rota-vapor at 40°C to have the crude extracts that were eventually dried at 45°C in an oven for 24 h.

Aqueous extraction

In 1500 mL of distilled water, 500 grams of the dry powders from the leaves and fruits of *P. sylvestris* were macerated for 48 h. After filtration, the macerate was concentrated overnight at 40°C in a rota-vapor to have the useful crude extract that eventually underwent accelerated drying at 45°C in an oven for 24 h.

Phytochemical screening

The phytochemical screening for secondary metabolites was carried out according to Zintchem *et al.*[12] and Bruneton[13] with a gallery of tests. The phytochemical derivatives targeted consisted of polyphenols, flavonoids, alkaloids, anthocyanins, coumarins, saponins, tannins, triterpenes, steroids, quinones and reducing sugars (table 1). Each extract characterization was performed with 2 mL of 1% extract solution (prepared in distilled water).

Table 1: Summary of phytochemical screening protocols.

Tests	Reagent added to extract solution and control solution (distilled water)	Indicator or positive reaction
Perchloride test (for polyphenols)	1% $FeCl_3$ (5 drops)	Development of a blue, violet, purple, green, or red-brown color
Leather acetate test (for polyphenols)	10% leather acetate (3 drops)	Development of a whitish precipitate
Test for Coumarins	10% $FeCl_3$ (3 drops) then HNO_3 (3 drops)	Color change: Extract color → blue or green color → yellow color
Sodium hydroxide test (for flavonoids)	1N NaOH (1 mL)	Development of a yellow-orange color who can change into colorless upon addition of a few drops of

		HCl solution
Shinoda test (for flavonoids)	Shinoda reagent/cyanidin reaction (5 drops)	Development of pink-orange, red, pink-purple color
Mayer test (for alkaloids)	Valse Mayer reagent (5 drops)	Development of a whitish-yellow creamy precipitate
Hager test (for alkaloids)	Hager reagent (5 drops)	Development of a reddish precipitate
Wagner test (for alkaloids)	Wagner reagent (5 drops)	Development of a whitish creamy precipitate
Test for anthocyans	Concentrated H_2SO_4 (2 mL) solution then ammonia (3 drops)	Color change: Extract color → a pink-red color → purple blue color
Frothing test (for saponins)	Distilled water (2 mL) then test tube was stopped and shaken vigorously for about 15 seconds.	Allowed to stand for 15 min, persistent frothing indicated the presence of saponins
Test for tannins	10% $FeCl_3$ (5 drops)	Development of an blue or green color
Test for triterpenoids	Chloroform (2 mL) then concentrated H_2S0_4 solution (3 mL) gently added to form a layer then test tube was heated for 120s in a water bath (65°C)	Development of a reddish brown coloration at the interface
Test for quinones	Concentrated H_2SO_4 (2 mL)	Color changing into reddish
Test for steroids	Chloroform (2 mL) then acetic anhydride (2 mL) then concentrated H_2SO_4 solution (2 drops)	Reaction order: development of reddish, then bluish and finally greenish color
Test for reducing sugars	Fehling solutions then test tube was heated (≈50°C) for 10 minutes	Development of a brick-reddish color

Antimicrobial potential of extracts

The microbial types used in this survey included *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus hominis*, *Streptococcus pneumoniae*, *Candida albicans*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton interdigitales*, *Trichophyton mentagrophytes*. Bacterial and *C. albicans* were provided by the Laboratory of Microbiology at the "Université des Montagnes" Teaching Hospital, while strains of *Trichophyton* species were obtained from the Laboratory of Microbiology, University of Douala.

Sterilization and sterility test of the extract solution

Before each step throughout the investigation of antimicrobial potential, extract solutions were prepared in sterile distilled water. These solutions were sterilized by filtration through a 0.22 µm-Millipore sterile membrane. Sterility was then attested by plating the resulting filtrates on Mueller Hinton agar and 72 h incubation at 37°C for bacteria. For *Candida* and for other fungi, this was done on Sabouraud/Chloramphenicol agar at 30°C for 72 h and 3

weeks, respectively. Sterility was attested by the absence of microbial growth after completion of incubation.

Confirmation of antibacterial and antifungal potential of the extracts

Tests were carried out by agar diffusion according to the "Comité de l'Antibiogramme de la Société Française de Microbiologie 2014"[14] with slight modifications.

For each strain, a few colonies from a 24 h subcultures were used to prepare a microbial suspension in 3 mL of sterile physiological saline to a turbidity scale similar to the one observed at the 0.5 standard of the McFarland's. Subsequently, this preparation was used to inoculate Mueller Hinton or Sabouraud/Chloramphenicol (5%) agar in Petri dishes (Φ=90 mm) by swabbing. To a sterile Whatman N°3 paper disc (Φ= 6 mm) firmly adjusted with sterile forceps on the plated preparation, 15 µL of the sterile extract solution at 50 mg/mL was dispensed. These preparations were thereafter incubated overnight at 37°C for bacteria and 30°C for *C. albicans*; and 7 days at 30°C for others fungi. Development of an inhibitory zone around the paper disk upon incubation indicated a positive reaction attesting the antibacterial or antifungal potential of extract.

Minimum Inhibitory Concentration (MIC) determination

The technique used was macro-dilution in appropriate broth.[15] For this, test tubes containing 1 mL of Mueller Hinton broth (for bacteria) or Sabouraud broth (for fungi) were used. These tubes were grouped into batches of 39. Each batch was used to assess in triplicate the MIC value of the extract for each microorganism.

In more details, 10 test tubes were used for a serial dilution (1/2) from the original (sterile extract solution at 100 mg/mL) through 50 mg/mL and 0.098 mg/mL of extracts. Then, 15 µL of a bacterial or fungal inoculum prepared as above developed was dispensed in each of the tubes within 15 min post-preparation. The inoculated suspensions were, thereafter, incubated for 24 h at convenient bacteria and *Candida* growth's temperature (37°C and 30°C, respectively) and 7 days at 30°C for *Trichophyton* spp. To each series, a "positive control" tube containing the broth and 15 µL of the microbial inoculum, a "negative control" tube made up of the broth and the 50 mg/mL extract, and a "sterility control" tube containing only the broth prepared during the whole procedure were added.

Subsequent to incubation, bacterial or fungal growth was assessed with naked eyes and confirmed after three independent identical reading results. In each set of test tubes, the lowest concentration of extract at which the medium remained non-turbid was recorded as the MIC for the extract on the test microorganism. Each result was validated when the bacterial or fungal growth was observed in the positive control tube while in the negative control and sterility controls, no growth was recorded.

Minimum bactericidal (MBC) and fungicidal (MFC) concentrations

They were determined by sub-culturing, 5 µL from the MIC preparations in which no microbial growth was recorded on Mueller Hinton or Sabouraud/Chloramphenicol agar. Positive and negative controls were added for all series. These preparations were, once again, incubated overnight at 37°C for bacteria, 30°C for *C. albicans* and 7 days at 30°C for *Trichophyton* spp.

Upon completion of incubation, microbial growth was assessed. For each extract, the MBCs and MFCs were defined at the lowest dilutions at which no visible bacterial or fungal growth was recorded on the culture agar. As in previous steps, each result was validated when the bacterial or fungal growth was observed in the positive control subculture while no growth was recorded in the negative control.

For MBC and MFC greater than 50 mg/mL, this process was repeated after a new serial microbial culture performed in liquid medium respecting a dilution of extract from 1.600 mg/mL to 50 mg/mL.

Inhibitory zone diameters at MLCs

For this essay, the protocol used to confirm antibacterial and antifungal potential of the extracts was repeated with slight modifications. Extract concentrations used were the MBCs and MFCs. For each extract concentration, the test was conducted in triplicate. After incubation, inhibitory zone diameters around the paper disk were measured.

Extracts' activity definition

Subsequent to the minimal inhibitor concentrations and minimal lethal concentrations (MLC), the MLC/MIC ratios were determined and used to categorize extracts' activity.[16] The extract was regarded as bactericidal when this ratio was lower than 4 and bacteriostatic

in the opposite situation. Fungicidal and fungistatic activity of extracts was respectively defined as done with bacteria.

Data analysis

The data were recorded and analyzed with tools provided by Microsoft Office Excel 2013 software.

RESULTS

Phytochemical screening of extracts

With regards to extracts' composition in secondary metabolites, it was observed that they contained various phytochemical groups. Major related findings were then brought together and summarized as displayed in table 2.

Table 2: Phytochemical composition of extracts.

Phytochemical groups	Hydro-ethanol extracts of *P. sylvestris*		Aqueous extracts of *P. sylvestris*		Hydro-ethanol extracts of *S. barbata*
	Leaves	Fruits	Leaves	Fruits	
Polyphenols	+	+	-	-	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	-
Anthocyanins	+	+	-	-	+
Coumarins	-	-	-	-	-
Saponins	+	+	-	-	+
Tannins	+	+	-	-	+
Triterpenes	+	+	+	+	+
Steroids	+	+	+	+	+
Quinones	-	-	-	-	+
Reducing sugars	+	+	+	+	+

–: Absent. +: Present

Overall findings revealed the presence of flavonoids, reducing sugars, triterpenes and steroids in all extracts while coumarins were not detected in any. The leaves and fruit of *P. sylvestris* displayed similar phytochemical composition with the one recorded with *S. barbata* when extraction was conducted with the hydro-ethanol procedure. The phytochemical composition was the same from leaves and fruits in each extract type. However, hydro-ethanol extracts presented much bigger diversity of target chemicals than aqueous extracts.

Antibacterial and antifungal potentials of the extracts

The confirmation tests of antibacterial and antifungal potentials revealed an antimicrobial activity on test strains.

Minimal inhibitory concentrations

Essays for the minimal inhibitory concentrations (MICs) of these extracts yielded pieces of information contained in table 3 for bacteria and table 4 for fungi.

Table 3: Minimal inhibitory concentrations (mg/mL) of extracts on bacteria isolates.

Extracts	*P. aeruginaosa*	*S. aureus*	*S. hominis*	*S. pneumoniae*	*K. pneumoniae*
$HE_{70°}E$-L *P. sylvestris*	12.5	25	50	6.25	6.25
$HE_{70°}E$-F *P. sylvestris*	50	50	50	50	25
AE-L *P. sylvestris*	12.5	12.5	12.5	25	12.5
AE-F *P. sylvestris*	12.5	12.5	25	12.5	12.5
$HE_{70°}E$ *S. barbata*	0.39	0.39	1.56	0.195	0.78
$HE_{70°}E$: hydro-ethanol extract; $HE_{70°}E$-L: hydro-ethanol extract of leaves; $HE_{70°}E$-F: hydro-ethanol extract of fruits; AE-L: aqueous extract of leaves; AE-F: aqueous extract of fruits					

Table 4: Minimal inhibitory concentrations (mg/mL) of extracts on fungal isolates.

Extracts	*C. albicans*	*T. rubrum*	*T. mentagrophytes*	*T. torsurans*	*T. interdigitale*
$HE_{70°}E$-L *P. sylvestris*	50	50	25	12.5	50
$HE_{70°}E$-F *P. sylvestris*	50	50	50	50	12.5
AE-L *P. sylvestris*	12.5	6.25	12.5	6.25	6.25
AE-F *P. sylvestris*	12.5	12.5	12.5	12.5	12.5
$HE_{70°}E$ *S. barbata*	0.39	0.39	0.78	0.78	0.195
$HE_{70°}E$: hydro-ethanol extract.; $HE_{70°}E$-L: hydro-ethanol extract of leaves; $HE_{70°}E$-F: hydro-ethanol extract of fruits; AE-L: aqueous extract of leaves; AE-F: aqueous extract of fruits					

The hydro-ethanol extract of the whole plant of *S. barbata* acted with the lowest MICs. They were followed respectively by the aqueous extract of *P. sylvestris* leaves and fruits. The inhibitory potential decreased from the hydro-ethanol extract of *S. barbata* to the aqueous extract of the leaves and fruits of *P. sylvestris* and the hydro-ethanol extract of the leaves and fruits of *P. sylvestris.* Overall, and regarding the lowest MICs, fungi appeared to be more vulnerable to extract effects than bacteria.

Minimal lethal concentrations and inhibitory zone

When the MLCs of the extracts were associated with related inhibitory zone diameters, summary of findings was done and presented in table 5.

Table 5: Minimal lethal concentration (mg/mL) and inhibitory zone diameters.

Microorganisms	MLC (mg/mL) of extracts Φ (mm)				
	$HE_{70°}E$-L *P. sylvestris*	**$HE_{70°}E$-F *P. sylvestris***	**AE-L *P. sylvestris***	**AE-F *P. sylvestris***	**$HE_{70°}E$ *S. barbata***
Pseudomonas aeruginosa	200 10	100 12	200 11	200 11	25 12
Staphylococcus aureus	200 13	200 14	200 12	50 13	100 16
Staphylococcus hominis	200 11	200 12	200 9	200 13	50 14
Streptococcus pneumoniae	200 13	200 13	200 10	100 10	50 14
Klebsiella pneumoniae	200 10	100 11	200 11	100 10	25 12
Candida albicans	400 10	100 16	400 11	100 10	100 15
Trichophyton rubrum	100 19	200 18	100 13	50 9	50 19
Trichophyton mentagrophytes	100 12	100 19	100 12	50 12	25 18
Trichophyton tonsurans	200 15	100 15	200 15	50 11	25 16
Trichophyton interdigitales	200 11	200 14	200 10	50 10	25 21

$HE_{70°}E$: hydro-ethanol extract; $HE_{70°}E$-L: hydro-ethanol extract of leaves; $HE_{70°}E$-F: hydro-ethanol extract of fruits; AE-L: aqueous extract of leaves; AE-F: aqueous extract of fruits

The highest MLCs were observed with the extract from *P. sylvestris* while the larger diameter and the lowest MLCs were recorded with *S. barbata* extract. The highest MLC value was observed on *C. albicans* and the lowest on *T. mentagrophytes*. With this extract in addition, the MLC values globally appeared to be lower on fungi.

Extracts' activity definition

To further characterize the extracts, the MICs and MLCs values were used to specify MLC/MIC ration as displayed in table 6.

Table 6: MLC/MIC ratios.

Extracts	$HE_{70°}E$-L *P. sylvestris*	$HE_{70°}E$-F *P. sylvestris*	AE-L *P. sylvestris*	AE-F *P. sylvestris*	$HE_{70°}E$ *S. barbata*
P. aeruginaosa	16	2	16	16	64
S. aureus	8	4	16	4	256
S. hominis	4	4	16	8	32
S. pneumoniae	32	4	8	8	256
K. pneumoniae	32	4	16	8	32
C. albicans	8	2	32	8	256
T. rubrum	2	4	16	4	128
T. mentagrophytes	4	2	8	4	32
T. torsurans	16	2	32	4	32
T. interdigitale	4	16	32	4	128

$HE_{70°}E$: hydro-ethanol extract; $HE_{70°}E$-L: hydro-ethanol extract of leaves; $HE_{70°}E$-F: hydro-ethanol extract of fruits; AE-L: aqueous extract of leaves; AE-F: aqueous extract of fruits

Overall, the extracts expressed both bacteriostatic and fungistatic activities. The bactericidal potential was observed in 4% of cases and the fungicidal in 16%. The highest bacteriostatic/fungistatic activities was recorded with the hydro-ethanol extract of *S. barbata* while the lowest bacteriostatic and fungistatic potentials were observed with the hydro-ethanol extract of *P. sylvestris*.

DISCUSSION

The present investigation aimed at assessing the antibacterial and antifungal potentials of extracts of the whole *Setaria barbata* (*Poaceae*) plant and; of leaves and fruits from *Pinus sylvestris* (*Pinaceae*). It focused on the hydro-alcoholic extract for both plants and the aqueous extract for *P. sylvestris*.

The phytochemical screening revealed the presence of target secondary metabolites in extracts with the larger phytochemical diversity in the hydro-ethanol extracts. Namely (but not limited to), they included flavonoids, triterpenes, reducing sugars, steroids, polyphenols, anthocyanins, saponins, tannins and alkaloids. Coumarins were not detected in these extracts. These findings were similar to those previously reported by Leau (2017)[17] who observed that the bud of *P. sylvestris* contained monoterpenes (α- and β-pinene, limonene), sesquiterpenes (β-caryophillene), polyphenols (pinosylvin and its derivatives), flavonoids (luteol derivatives, 5-methoxysalvigenol, alpigenol), tannins (proanthocyanidols), esters (bornyl acetate), Oleoresin (terebenthine) and maltol. Further in the present investigation, the aqueous extracts of leaves and fruits of *P. sylvestris* did not contain anthocyanins, polyphenols, saponins and tannins. This difference in chemical composition between the two

extracts of *P. sylvestris*, is most likely in connection with the extraction protocols as suggested by Thagara *et al*.[18] This point of view is shared by several previous works including those conducted by Moroh *et al*. (2008) and Bagré *et al*. (2011) who observed that ethanol was more suitable for extraction than distilled water.[19,20] The absence of coumarins and quinones in the leaves and fruit extracts of *P. sylvestris* and even, in the essential oils described by Leau *et al*. (2017)[17] indirectly indicates either low concentration or absence of these metabolites in this plant.

Regarding their antibacterial and antifungal potentials, it was observed that at 50 mg/mL, all extracts were active on target microorganisms. This finding could be anticipated with regards to the phytochemical compounds detected which are known for their antimicrobial properties.[21,22,23]

Subsequent investigations through the MICs indicated the lowest value with the hydro-ethanol extract of *S. barbata* (0.16 – 1.56 mg/mL) followed by the aqueous extract of the leaves of *P. sylvestris* (6.25 – 25 mg/mL) and the aqueous extract of the fruits of *P. sylvestris* (12.5 – 25 mg/ml). With the hydro-ethanol extracts of *P. sylvestris* leaves and fruits, higher MICs values were recorded (6.25 to 50 mg/mL for the leaves and 12.5 to 50 mg/mL for the fruits). Globally, the lowest MICs were observed with fungi. As observed by previous authors and consistent with above development, extract potential depends on the plant species, the method of extract preparation, the chemical composition of the extracts and the tolerance of the microorganisms.[24,25,26] The susceptibility/resistance profile to conventional drugs of the test microorganisms were not investigated, but it could play a key role in developing these discussions sustainably. Furthermore, the chemical composition and concentration of active compounds/secondary metabolites depend on environmental determinants like stresses, climate and the chemical composition of the soil.

The best inhibitory potentials decreased from the hydro-ethanol extract of the whole plant of *S. barbata*, through the aqueous extract of the leaves of *P. sylvestris* to the aqueous extract of the fruits of *P. sylvestris*. If this trend could be predicted based on the phytochemical composition of the *S. barbata* extract, it couldn't be the case for the *P. sylvestris* extracts. Accordingly, knowledge of the phytochemical composition of an extract based on the presence or absence of one or more compounds could help predict whether a plant possesses antimicrobial potential or not; but is not enough to accurately predict antimicrobial activity or to anticipate that one plant or extract could express better inhibitory ability than the other.

Amongst other explanation attempts, interactions amongst identified chemicals remain unknown; just as the actions of other chemicals that are not investigated in specific studies. These findings raise several reasonable hypotheses that should be taken into consideration in future investigations. One of these hypotheses would be that the hydro-ethanol extracts contain higher phytochemical diversity which are low in their concentration, compared to the aqueous extracts characterized by lower phytochemical diversity and higher inhibitory potentials. Another one would be the presence of antagonistic or limiting action of one or more identified and/or unidentified metabolites as developed above. Still another one might be in connection with the variable tolerance of the microorganisms based on their natural behavior and/or on antibiotic susceptibility/resistance profile that could serve as landmark of comparative studies for future substitution in the production of alternative drugs; or other determinants that could have generated other results. Data on the MLCs and inhibitory zone diameters were associated in most cases. The MICs and MLCs values were slightly proportional. Also, the hydro-ethanol extract of *S. barbata* displayed the highest lethal potential on the tested microorganisms though this potential was lower for the fruits and the leaves *P. sylvestris* extracts. Arguments used to explain MIC results could also explain results for the MLCs and inhibitory zone diameters. Concerning the extracts' activity, it was observed that the extracts express a bacteriostatic potential in 96% and a fungistatic activity in 84% of cases. Bacteriostatic/fungistatic nature of hydro-ethanol extract of *S. barbata* were higher than other extracts. After *S. barbata* extract, bacteriostatic and fungistatic potentials decreased with the aqueous extract of *P. sylvestris* leaves, the hydro-ethanol extract of *P. sylvestris* leaves, the aqueous extract of *P. sylvestris* fruit and the hydro-ethanol extract of *P. sylvestris* fruits, respectively. Similar developments on the MICs could be afforded as explanation to the extracts' potentials recorded throughout the study.

Above findings are clues that support the use of these plants resources by traditional healers and could generate new issues into the development of alternatives for conventional anti-infective agents in low- and middle-income communities in which raw material is available, in line with the WHO. But minimal research, political will and holistic facilities should be promoted and provided for optimal use of previous and current knowledge on plant resources. Amongst the actions to be carried out in this line, development of standards is primordial for accurate use of the improved herbal medicine that will otherwise, be available and affordable to larger numbers of needy populations. This view is crucial and should be fostered as the world populations are facing increased antibiotic resistant and new threats like influenza and

COVID-19 for which caretaking partly relies on mastering opportunistic infections and other comorbidities in connection with metabolic disorders for which plant secondary metabolites are known to have significant potentials. Critical thinking on this issue is paramount, acknowledging that no one would accurately anticipate the forthcoming threats.

CONCLUSION

The present survey on the antimicrobial potentials of extracts from *Pinus sylvestris*, and *Setaria barbata* revealed variable potentials likely associated with their chemical composition. The hydro-ethanol extracts were richer in the secondary metabolites investigated. *S. barbata* extract exhibited higher inhibitory and lethal potentials. For *P. sylvestris*, aqueous extracts exhibited higher inhibitory potentials and hydro-ethanol extracts higher lethal potentials. This study provides scientific justification for the use of the leaves and fruits of the *Pinus sylvestris* and the whole *Setaria barbata* plant by traditional healers, but minimal requirements are necessary for sustainable use of these plants in the control of infectious diseases.

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