Phytochemical Screening and Antimicrobial Activity of *Sarcocephalus latifolius* Smith Extracts

ABSTRACT

Aims: The aim of this work is to evaluate the antimicrobial activity of *Sarcocephalus latifolius* extracts.

Methodology: Thus, a phytochemical screening was qualitatively access using colorations or precipitations methods. Ethanolic and aqueous extracts were used to evaluate the antimicrobial activity. The antimicrobial activity, using diffusion method, was evaluated on eight strains including two reference strains (*Streptococcus pneumoniae* ATCC 49619 and *Pseudomonas aeruginosa* ATCC 27853) and six clinical isolated *S. pneumoniae* and *P. aeruginosa* strains. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined by the microdilution method.

Results: The phytochemical screening showed the presence of flavonoids, anthocyanins, mucilages, saponosides, C-heterosides and O-heterosides. Antimicrobial activity showed that the ethanolic extract with the lowest MIC (1.25 mg/ml) inhibited reference strains (*S. pneumoniae* ATCC 49619 and *P. aeruginosa* ATCC 27853) and clinical isolated *S. pneumoniae* and *P. aeruginosa* strains. The largest inhibition diameter (19± 1.33) was obtained with the ethanolic extract against clinical isolated *Pseudomonas aeruginosa* and (15.5± 1) against the reference one. The aqueous extract inhibited only reference strains.

Conclusions: The data of this study indicate that the extracts of *S. latifolius* present antimicrobial properties. This may justify its traditional use in treatment of microbial infections.

Keywords: phytochemical Screening*, Antimicrobial activity, Sarcocephalus latifolius, Benin*

1. INTRODUCTION

Medicinal plants occupy an important place in African pharmacopoeia [1]. Research on medicinal plants has intensified due to the diverse therapeutic potentials that these medicinal plants possess. The evaluation of plants in traditional medicine gives us clues on how these plant parts can be used as antimicrobial agents against many pathogens [2]. The use of plant extracts and physicochemical both known for their antimicrobial properties can be of great importance in therapeutic treatments [3]. Many plants have been used because of their antimicrobial characteristics that are due to their secondary metabolites contain. *Sarcocephalus latifolius Smith* (Rubiaceae) is used in many African countries by traditional medicine practitioners for the treatment of various ailments including bacterial diseases [4].

In Africa, *S. latifolius* is widely used in traditional medicine to treat a variety disease including malaria, epilepsy and infectious diseases [4], dysentery and diarrhea [5], hernia, ascites, vomiting and colic [6]. In addition, good *in-vitro* antioxidant, anti-inflammatory and anti-diabetic effects of this plant leaf and fruit extracts have also been reported [7,8].

In Benin, infectious diseases are the primary public health problem [9]. These infectious diseases are often caused by microbial pathogens. To control the pathogens involved in the infectious diseases, antibiotic therapy is implemented currently used [10]. Unfortunately, the resistance phenomenon is increasing cause of treatment failure. One of the options remains to find a local and natural, such as the uses of plants, solution to mitigate these health problems.

Among the potential plant, *S. latifolius* has been identified and used due to its medicinal properties regarding gastric disorders and foodborne diseases [11]. This is proof that traditional medicine still has unexplored potential. However, the main problem with traditional treatments, especially those based on plants, is the lack of scientific knowledge regarding efficacy, mode of action, active ingredients, doses to be administered, indications, lack of properties, safety and quality control. Therefore, the present study aimed to evaluate the phytochemical component and antioxidant potential of *S. latifolius* extracts.

2. MATERIAL AND METHODS

2.1. Samples collection and pulverization

Once collected from the surveyed area, the roots of *Sarcocephalus* latifolius were certified at the National Herbarium of Benin under the identification number YH687/HNB. The collected samples were then clean and dried for about 14 days in to laboratory room temperature (22±2°C). After drying, roots samples were powdered (Retsch mill SM 2000/1430/Upm/Smf) and stored until used for the different activities.

2.2. Samples analysis

2.2.1. Preliminary phytochemical screening

The qualitative analysis of preliminary phytochemical screening was performed directly on the plant root powder using the adapted method of Houghton and Raman [12].

2.2.2. Obtaining the extracts

Ethanolic and aqueous extracts obtained according to a previously developed method [13] were used in this study. The choice of these types of extracts is based on the way the plant is traditionally used. For the aqueous extracts, 50 g of obtained powder were macerated in 500 ml of distilled water for 72 hours under continuous stirring. The obtained homogenate was successively filtered three times on absorbent cotton and once on Whatman paper. This filtrate was then dried in an oven at 50°C and the powder obtained constitutes the total aqueous extract. Concerning the ethanolic, 50 g of powder was macerated in 500 ml of 96% ethanol for 72 hours under continuous stirring. The mixture was then filtered three times on absorbent cotton and once on Whatman n°1 to obtain a solids-free solution. The filtrate, was concentrated in a rotary evaporator at 50° and stored at 2-4°C.

The extraction yield is defined as the ratio of the mass of dry extract obtained to the mass of plant material processed [14]. It was obtained according to the following formula: $R (\%) = (Me/Mv) \times 100$ with R (%): yield in %, Me: mass of dry extract, Mv: Mass of plant material used.

2.3. Antibacterial activity

2.3.1. Sensitivity test

The *in-vitro* antibacterial activity of extracts was demonstrated by solid medium diffusion method with the use of Whatman N°1 paper as previously described by Chabi Sika et al. [13]. Thus, 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 Mc Farland scale (10^8 CFU/ml) and reduced to 10^6 CFU/ml in sterile distilled water. This bacterial suspension (100 µl) is used to flood a petri dish containing Mueller-Hinton agar (Bio Rad, France). The sterile discs (6 mm) were deposited, under aseptic conditions, on plates previously 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 being incubated at 37°C in the oven for 24 h and 48 h. Inhibition diameters are measured with a graduated ruler after incubation times of 24 h and 48 h.

2.3.2. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the extracts were determined following the microdilution method using iodonitrotetrazolium (INT) as a viability indicator for bacteria [15]. A range of nine concentrations (10 to 0.039 mg/ml) of the extracts was tested on the microbial strains. Then, 150 µl of bacterial inoculum (10^6 CFU/ml) was added to all wells. The plates were then incubated at 37°C. After 18 h of incubation, 10 µl of INT (0.2 mg/ml) was added to all wells. Plates were re-incubated at 37°C for 30 min. The MIC corresponds to the first well in which no red/pink coloration due to the presence of INT is observed.

2.3.3. Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined on the basis of the results of the MIC determination. To do this, after identifying the MIC, we used a loop to inoculate all the other wells from the MIC to the high concentrations on petri dishes containing MH agar medium. 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 MBC [16].

The antibacterial effect was considered bactericidal or bacteriostatic depending on the MBC/MIC ratio [17]. Thus, the interpretation of the results is reflected in the ranges below: i- MBC/MIC≤ 4 (bactericidal effect) and ii- MBC/MIC > 4 (bacteriostatic effect).

2.4. Data Analysis

Acquired data were analyses using GraphPad Prism 8 software. For each extract, the lethal concentration that causes 50% larval death (LC_{50}) was calculate 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 (LC_{50}) corresponding to the death of half the larvae.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The results of the phytochemical study of the root of *Sarcocephalus latifolius* are presented in Table 1. We note a strong presence of flavonoids and an average presence of anthocyanins, mucilages, saponosides, C-heterosides and O-heterosides with reduced genine. On the other hand, we note the absence of catechic tannins, gall tannins, leucoanthocyanins, alkaloids, reducing compounds, cyanogenic derivatives, triterpenes, steroids, coumarins, quinonic derivatives, free anthracene, O-heterosides and cardiotonic derivatives.

Table 1. Families of secondary metabolites sought in the root of *Sarcocephalus latifolius*

Secondary metabolites	Results
Gallic tannins	-

Catechic tannins	-
Flavonoids	++
Leucoanthocyanins	-
Anthocyanin	+
Alkaloids	-
Reducing compounds	-
Mucilage	+
Saponosides	+
Cyanogenic derivatives	-
Terpenes	-
Steroids	-
Coumarin	-
Quinones derivatives	-
Free anthracenic	-
C-Heterosides	+
O-Heterosides	-
O- Heteroside with reduced genius	+
Cardiotonic heterosides	-

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

3.2. Extraction yield

Analysis of the extraction yield with both solvents (Figure 1) showed that the yield of the aqueous extract (9.28%) was higher than that of the ethanolic extract (3.54%). Thus, water concentrated the secondary metabolites contained in *Sarcocephalus latifolius* root better compared to ethanol.

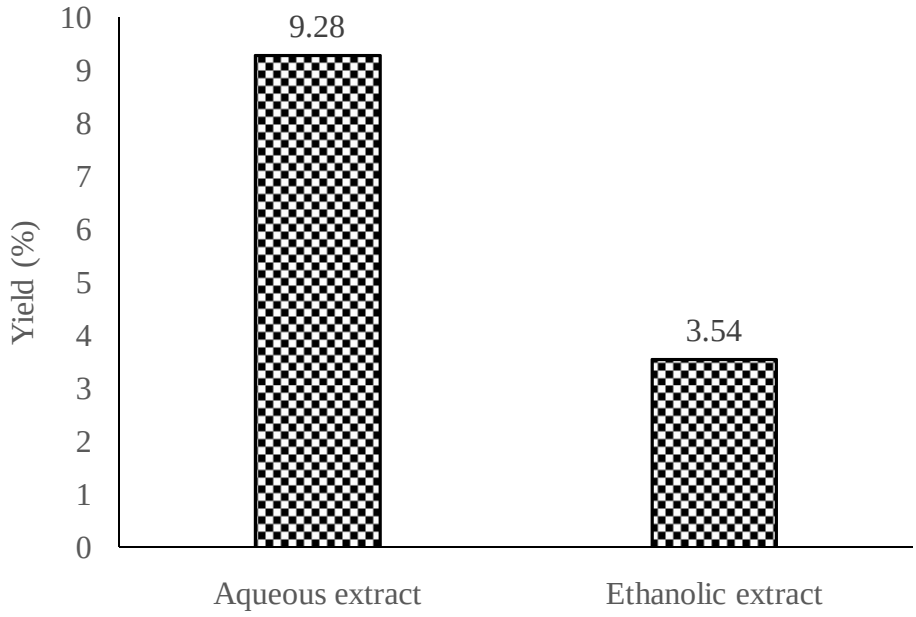

Figure 1. Yield of the prepared extracts

3.3. Antimicrobial activity

3.3.1. Sensitivity test

Table 4 presents the inhibitory activity of *Sarcocephalus latifolius* extracts. It appears from its analysis that the ethanolic extract has a higher inhibitory activity against the tested microorganisms than the aqueous extract. Also, the ethanolic extract shows a wide spectrum of antimicrobial activity against clinical strains. Moreover, the largest inhibition diameter was obtained with the ethanolic extract (19± 1.33) against *clinical Pseudomonas aeruginosa* and (15.5± 1) against the reference one. Thus, the inhibition diameter varies with the species. The reference strains are sensitive to the aqueous extract in 24 h but the clinical strains are resistant to this extract.

Table 2. Inhibitory activity of *Sarcocephalus latifolius* extracts against strains

Tested strains	Aqueous extract		Ethanolic extract	
	24 Hours	**48 Hours**	**24 Hours**	**48 Hours**
S. pneumoniae ATCC49619	10.5**±**0.5	0	12**±2**	**9±**1
P. aeruginosa ATCC27853	11±1	0	15.5±1	**7±**2
Clinical isolated S. pneumoniae	0	0	15±1.33	15.17±0.94
Clinical isolated P. aeruginosa	0	0	19±1.33	14.33±4.22

Figure 2 presents the sensitivity of clinical strains of *Streptococcus pneumoniae* to the extracts and reveals that within 24h and 48h, the clinical strains of *Streptococcus pneumoniae* are sensitive to the ethanolic extract and resistant to the aqueous extract.

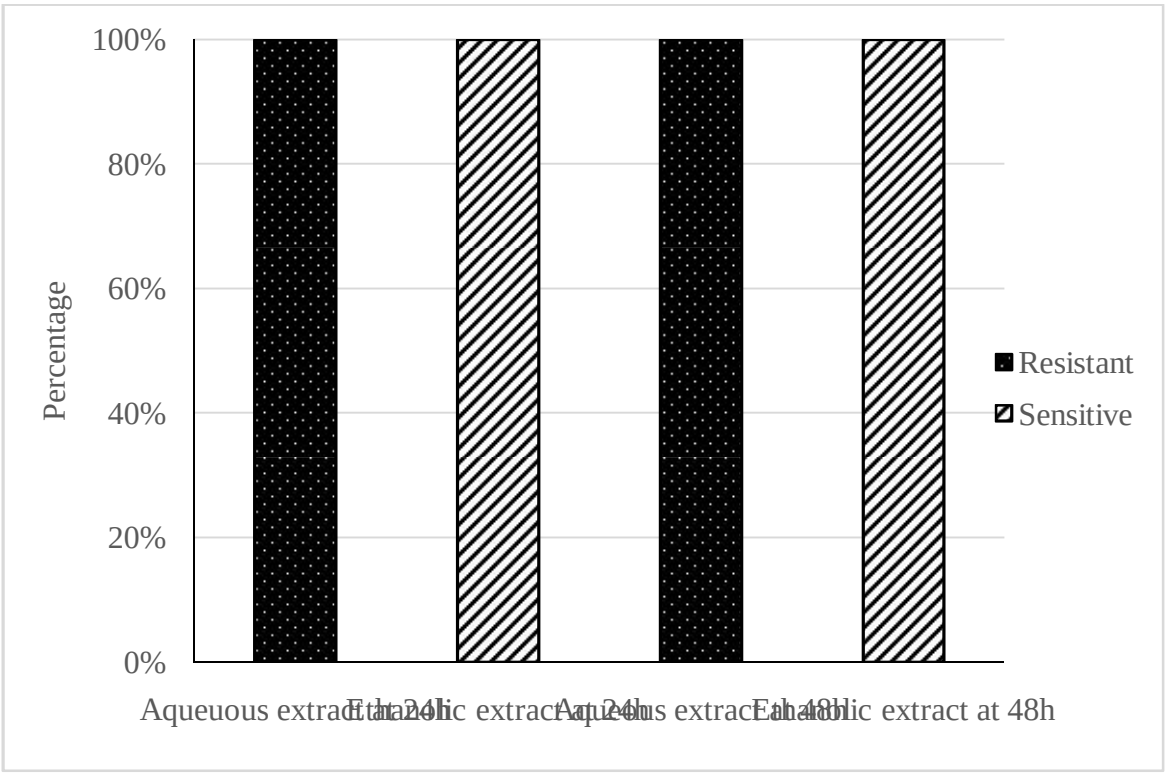

Figure 2. Sensitivity of *Streptococcus pneumoniae* strains to *Sarcocephalus latifolius* extracts

Figure 3 presents the sensitivity of clinical strains of *Pseudomonas aeruginosa* to extracts and reveals that within 24 and 48 hours, the strains of *Pseudomonas aeruginosa* are sensitive to ethanolic extract and resistant to aqueous extract.

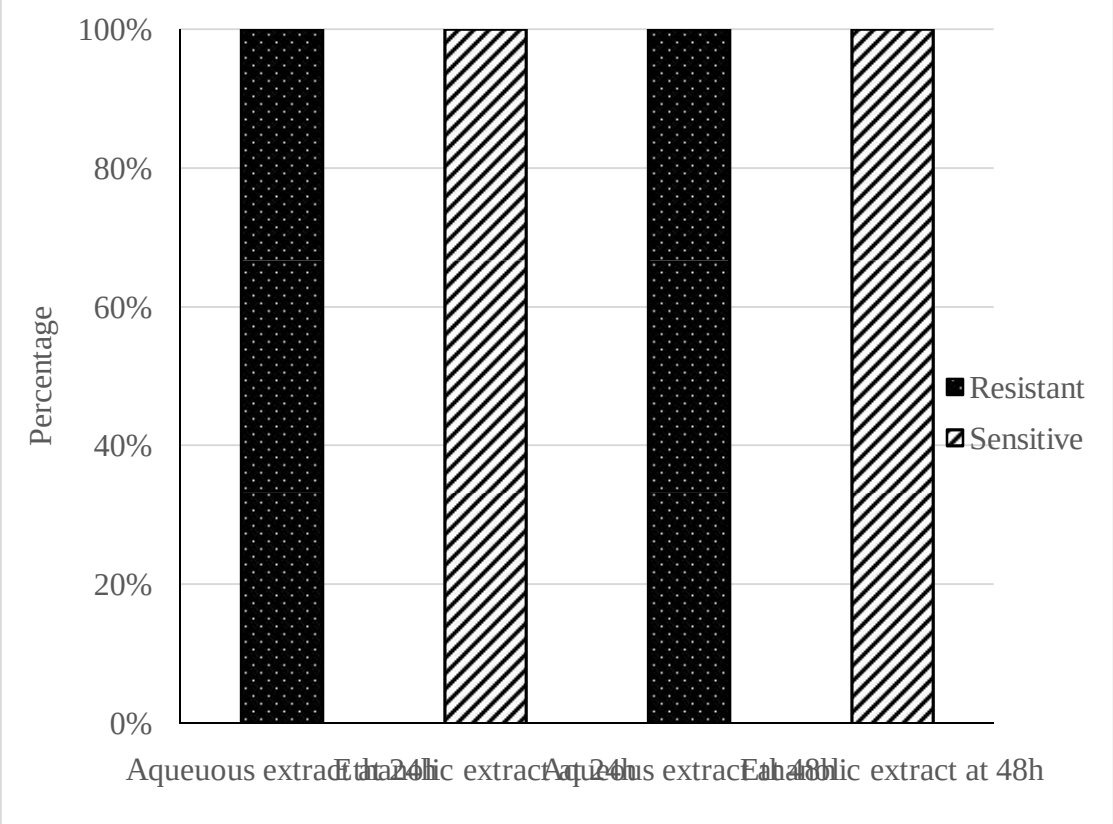

Figure 3. Sensitivity of *Pseudomonas aeruginosa* strains to *Sarcocephalus latifolius* root extracts

3.3.2. Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

Table 3 presents the Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration of the two extracts on the strains studied. It appears from its analysis that the aqueous extract presented MICs of 2.5 and 5 mg/ml against the reference strains while the MICs of the ethanolic extract vary from 1.25 and 2.5mg/ml. Regarding the BMC, they are 10mg/ml for the aqueous extract against the reference strains and vary from 5 to 10 mg/ml for the ethanolic extract. According to the BMC/MIC ratio, we notice that all the extracts have a bactericidal effect on all the tested strains.

Table 3. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the extracts of the three plants on the strains studied.

Strains	CMI et CMB (mg/ml) of *Sarcocephalus latifolius* extracts							
	Aqueous extract				**Ethanolic extract**			
	CMI	**CMB**	**CMB/CMI**	**Nature of activity**	**CMI**	**CMB**	**CMB/CMI**	**Nature of activity**
S. pneumoniae ATCC49619	5	10	2	Bactericidal	2.5	10	4	Bactericidal

P. aeruginosa ATCC27853	2.5	10	4	Bactericidal	2.5	10	4	Bactericidal
Clinical S. pneumoniae	-	-	-	/	2.5	5	2	Bactericidal
Clinical P. aeruginosa	-	-	-	/	1.25	5	4	Bactericidal

4. DISCUSSION

Table 1 presents the families of secondary metabolites sought in the root of *S. latifolius*. From this table, it appears that the powder from the root of *S. latifolius* showed the presence of secondary metabolites with the desired antimicrobial and antioxidant properties. Phytochemical analysis reveals the presence of flavonoids, anthocyanins, mucilages, saponosides, C-heterosides and O-heterosides with reduced genin. These secondary metabolites, identified within this organ, are well known for their biological activities. The results obtained are similar to those of Ahoyo et al. [9] who found alkaloids, tannins, catechic tannins, gallic tannins, reducing compounds, steroids, triterpenes, quinone derivatives and coumarins in the root of *S. latifolius*. This may be due to the phenology of the species and also to the influence of several factors such as variation in genetic makeup, weather conditions, geographical location of the plants, the part of the plant studied and the method of extraction used [18, 19]. Flavonoids are recognized for their very broad and very diversified antibacterial activities, very powerful antifungals, antioxidants including their ability to scavenge free radicals. Also, saponosides are endowed with anti-inflammatory and antibacterial activity; which justifies the antimicrobial and antioxidant power of the roots of *S. latifolius*. Note the absence of cyanogenic derivatives and cardiotonic glycosides, which are toxic substances that would jeopardize its health safety and therefore promote its wide use in traditional medicine.

Regarding the yield, the aqueous extract produced the highest yield (9.28%) comparing to the ethanolic extract (3.54%). Similar reports were also reported by Ekong and Chijioke [20] in Nigeria on extracts of *S. latifolius* where the best yield was obtained with the aqueous extract (37.7%) compared to the ethanolic extract (31.0%). This could be explained by the fact that several parameters affect the extraction procedure such as the chemical form of the compounds studied, the extraction method, the size of the particles sampled, the parts of plants used, the polarity of the solvent, the conditions drying and extraction time [21].

The inhibitory activity of *Sarcocephalus latifolius* extracts against strains reveals that the ethanolic extract has a broad spectrum of antimicrobial activity against clinical *P. aeruginosa* strains with an inhibition diameter of 19 ± 1.33. These results are similar to those of Okwori et al. [22] who in Nigeria found that the ethanolic extract produced an average inhibitory zone ranging from 10 to 20 mm on *P. aeruginosa*. On the other hand, Ekong and Chijioke [20] proved that the aqueous root extract of *S. latifolius* better inhibits the growth of various strains where the best inhibition diameter was obtained *with P. aeruginosa* from bacteria cultures. This may be due to the physicochemical extraction capacity of ethanol. Also figures 3 and 4 indicate to us that in 24 and 48 h, the clinical strains of *S. pneumoniae* and *P. aeruginosa* are sensitive to the ethanolic extract and resistant to the aqueous extract in 48 h. This observed resistance could be due to natural resistance, genetic variability or mutational changes. The antimicrobial activity observed in the present study may be linked to the richness in bioactive metabolites, in particular flavonoid and saponoside. This plant could therefore be a better alternative in the effective fight against microbial infections caused by *S. pneumoniae* and *P. aeruginosa*.

The MICs obtained vary (from 1.25 to 5mg/ml) according to the types of strains and the type of extract. The lowest MIC (1.25mg/ml) was obtained with the ethanolic extract against the clinical strains of *P. aeruginosa* and the highest MIC (5mg/ml) with the aqueous extract against the reference strains of *S. pneumoniae*. We can therefore say that the ethanolic extract has a more effective action against this strain. These results are similar to those of Okwori et al. [22] who found an MIC of between 0.19 and 6.25 mg/ml with aqueous extracts

against strains of *Pseudomonas aeruginosa*. In addition, these results are contrary to those found by Ekong and Nnatu [20] when the reported that the MIC varies between 3.13 and 25mg/ml and the lowest MIC (3.13mg/ml) was obtained with the aqueous extract against strains of *Escherichia coli*. The differences observed between the values of our MICs and those of the authors cited above could be explained by the method of extraction, the solvents used and the plant organ and also the origin of the strains. Therefore, 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.

Considering the CMBs, they are 10mg/ml for the aqueous extract against the reference strains and vary from 5 to 10mg/ml for the ethanolic extract against the two types of strains. In addition, the extracts of this plant have a bactericidal activity on all the strains studied. These results corroborate those of Ekong and Nnatu [20] who showed the aqueous and ethanolic extracts of the root of *Nauclea latifolius* have a bactericidal effect on the strains tested. This will mean that extracts from the root of *S. latifolius* can be used as an antimicrobial agent in the treatment of bacterial infections. These results clearly indicate the meaning of their uses as an herbal remedy in the treatment of infectious diseases.

5. CONCLUSION

This work, with a view to confirming or invalidating the practice of medicinal plants, represents a step forward in the improvement of traditional medicine in general and in particular in the rational exploitation of *Sarcocephalus latifolius*. The results obtained showed that the phytochemical screening revealed the presence of compounds with antioxidant and antimicrobial activity. The evaluation of the antimicrobial activity showed that all the extracts have a bactericidal effect on the tested strains. In view of these results, the use of the root of *S. latifolius* in traditional medicine to treat pulmonary infections in general and pneumococcal diseases in particular is justified. However, additional studies such as antioxidant and toxicity are needed to demonstrate the efficacity and safety of *S. latifolius* root extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

K.C-S, H.S, B.B, I.M-S and L.B-M designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. **G.R. A.K, L.K, H.A.S, S.A.A and M.Y.A** managed the analyses of the study. '**K.C-S, I.M-S, B.B, L.K, and H.S** managed the literature searches. All authors read and approved the final manuscript.

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