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Phytochemistry, anti-radical and antimicrobial activities of *Sarcocephalus latifolius* leaves, used in Benin to treat urinary tract infections

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Abstract

Infectious diseases are a significant public health concern. To deal with antibiotic resistance, high costs and difficulties in accessing synthetic drugs, medicinal plants constitute a potential source. This study aims to valorize *Sarcocephalus latifolius* through the identification of its secondary metabolites and the evaluation of the antiradical and antimicrobial activities of its leaves. After the determination of secondary metabolites by the coloring and precipitation reactions, the contents of phenolic compounds were determined by the spectrophotometer. The antiradical and antimicrobial activities were evaluated respectively by the DPPH and Agar diffusion methods. According to the results obtained, the leaves of *Sarcocephalus latifolius* contain catechic tannins, saponosides, flavonoids, leuco an-thocyanins, coumarins, mucilages, sterols and terpenes. The total phenolic content is 85.97mgGAE/gEx with a total flavonoid content of 13.26mgQE/gEx and 9.19 mgEL/gEx for the total tannin content. The concentration of the hydroethanolic extract of this plant allowing 50% of the DPPH free radical to be trapped is 0.660 mg/mL. From 16 microbial strains tested, the hydroethanolic extract of *Sarcocephalus latifolius* leaves showed interesting antimicrobial activity against 14 strains with CMBs varying from one strain to another. This extract showed a broader spectrum of activity than the two synthetic antibiotics (ciprofloxacin and amoxicillin) used in this study.

Keywords: *Sarcocephalus latifolius*, infections, secondary metabolites, microbial strains.

1. Introduction

Urinary tract infections are among the most common bacterial infections worldwide with a wide spectrum of illnesses, ranging from simple cystitis to potentially life-threatening urosepsis [1]. These infections represent approximately 50% of nosocomial infections [2]. Infectious diseases remain one of the leading causes of death worldwide. They occur in any part of the urinary system. These diseases constitute a major public health problem [3]. Faced with bacterial resistance to antibiotics, high costs and difficulties in accessing synthetic drugs, it is imperative to increase investigations into medicinal plants to treat infections [4]. Traditional medicine plays a very

important role in providing primary health care to the population in developing countries [5]. Medicinal plants have been used for centuries to treat various pathologies arising from ailments. Many studies have been carried out over the last decades on plant species to discover their chemical composition and biological activities [6],[7], [8],[9],[10]. *Sarcocephalus latifolius* of the Rubiacae family is a shrub ranging from 5 m to 9 m high [11]. It has a rough bark. Its leaves are elliptical or rounded oval [11], [12]. *Sarcocephalus latifolius* is used to treat various conditions such as stomach disorders, gonorrhea, cough, fever, hemorrhoid, dysentery, malaria, bilharzia, headache, constipation, syphilic chancre, gonorrhea...[13],[14]. *Sarcocephalus latifolius* is also one of the plants that are heavily used in traditional medicine in Benin to treat urinary tract infections. It becomes necessary to direct work towards phytochemical and antimicrobial analyzes of the ethanolic extract of *Sarcocephalus latifolius* leaves.

2. Material and Methods

Material

Plant material: The plant material consists of leaves of *Sarcocephalus latifolius* collected in northern Benin.

Microbial strains: The microorganisms tested consisted of sixteen (16) bacterial strains (Staphylo-coccus aureus, *Escherichia coli*, *Escherichia coli* ATCC 25922, *Streptococcus D*, *Klebsiella pneumoniae*, *Streptococcus D*) and a fungal strain (*Candida albicans* MHMR). Bacterial strains are clinical strains isolated from urine, semen and vaginal swabs.

Methods

Collection of plant material: After the identification of the plant sample at the National Herbarium of Benin located at the University, they were dried in the laboratory until the plant mass stabilized before being reduced to powder.

Preparation of the hydroethanolic extract: The hydroethanolic extract was made by the maceration technique in the water-ethanol mixture (30/70). The macera obtained was filtered with Whatman paper. The filtrate obtained was then evaporated using a rotary evaporator. The concentrate was dried in an oven at 40°C until total evaporation [7]

Preliminary phytochemical screening: The secondary metabolites of *Sarcocephalus latifolius* were demonstrated by coloring and precipitation reactions specific to each secondary metabolite [9][15],[16],

Table 1: Methods for the identification of secondary metabolites of *Sarcocephalus latifolius* leaves

Secondary metabolites	Chemical test
Alkaloids	Mayer's test and Dragendroff's test
Anthocyanes	HCl and NH_3 test
Anthraquinones	Borntranger's test
Coumarins	UV 365 nm
Flavanoids	Shibita's reaction test
Tannins	Ferric chloride and sodium acetate test
Saponins	Frothing test
Leuco anthocyanins	Bate-Smith and metcalf
Mucilages	flaky test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test)

3. Dosage of phenolic compounds

Total phenols: The determination of total phenolic compounds was done using the Folin-Ciocalteu reagent [9], [17].

Total flavonoids: The aluminum trichloride ($AlCl_3$) method was used to quantify total flavonoids [17].

Condensed tannins: Condensed tannins are measured using the Butanol-HCl method. The reaction medium is composed of 0.5 mL of extract, 3 mL of butanol-HCl (95/5) and 0.1 mL of a ferric solution (2% ferric ammonium sulfate, diluted in 2N HCl). The samples are incubated in a boiling water bath for 60 min. The absorbance is measured at 550 nm and the results are expressed in leukoyanidine equivalents, according to the following formula: T (mgEL/gEx) = (A x 78.26 x FD); A: is the absorbance recorded at 550 nm; FD: the dilution factor. The dilution

factor is equal to 1 if the extract is prepared at 200 mg in 10 mL of solvent and the measured absorbance is less than 0.6 [18].

Antiradical activity: The antiradical activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The principle of this method is based on measuring the trapping of free radicals in a DPPH solution. This trapping is visualized by the disappearance of the purple color of the DPPH. The tanks are left in the dark for one hour and the absorbances were measured at 517nm [16], [19]. The percentage of radical trapping was determined by the formula:

$p=\frac{Ab-Ae}{Ab}$; *With P: trapping percentage; Ab: absorbance of the blank, Ae: Absorbance of the sample*

4. Antimicrobial activity

Sensitivity test: The sensitivity test of the different extracts obtained was demonstrated according to the method used by Chabi Sika *et al* [20] in solid Mueller Hinton (MH) medium. Indeed, a bacterial pre-culture (1 colony in 1 mL of liquid Mueller-Hinton) from the day before is diluted to obtain a turbidity of 0.5 on the McFarland scale (i.e. 10^8CFU/mL) and reduced to 10^6 CFU/mL in sterile distilled water. This bacterial suspension (1000 µL) is used to flood a petri dish containing Mueller-Hinton agar (Bio Rad, France). Using a perforator, 6 mm diameter paper discs were made. The sterile discs are placed, under aseptic conditions, on dishes formerly flooded with bacterial culture. On the deposited discs, 30 µL of extract to be tested are inoculated under aseptic conditions. For each extract, the experiment is duplicated and a negative control is carried out 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. The inhibition diameters are measured using a graduated ruler after incubation times of 24 h and 48 h.

Determination of minimum inhibitory and bactericidal or fungicidal concentrations

The Minimum Inhibitory and bactericidal or fungicidal Concentrations were determined by the micro dilution method using iodonitrotetrazolium as an indicator of viability of the microbial strains [21].

5. Results & Discussion

Preliminary phytochemical screening

Secondary metabolites identified in the leaves of *Sarcocephalus latifolius* are recorded in table 2. Phytochemical screening of leaves of *Sarcocephalus latifolius* revealed the presence of anthocyanins, mucilages, saponosides, leucoanthocyanins, catechic tannins, reducing compound, coumarins, sterols and terpenes. Badiaga [22] reported the presence of alkaloids for the Mali sample while the alkaloids were not found in the leaves of *Sarcocephalus latifolius* harvested in Benin. Similarly, Edewor *et al* [23] noted in the leaves of *Sarcocephalus latifolius* collected in Nigeria, the absence of anthraquinones which were identified for the species from Benin. On the other hand, our results corroborate those of Kamirou *et al* [14] at the level of the sample collected in Benin. The variation in secondary metabolites noted in our samples compared to previous work could be linked to the harvest period, the nature of the soil or climatic factors [22],[24],[25]. The diversity of secondary metabolites of these plants could explain their uses in the treatment of several conditions, namely tannins, flavonoids, coumarins, anthraquinones, sterols and triterpenes [26],[27],[28],[29].

Table 2: Secondary metabolites identified in *Sarcocephalus latifolius* leaves

Secondary metabolites	
Alkaloids	Absent
Flavonoids	Present
Anthocyanins	Present
Leuco anthocyanins	Present
Reducing compounds	Present
Tannins	Present
Sterols and terpenes	Present
Mucilages	Present
Saponosides	Present
Coumarins	Present
Cyanogenic derivatives	Absent

Content of total phenols, total flavonoids and condensed tannins of *Sarcocephalus latifolius* extracts

Table 3 presents the contents of total phenolic compounds, expressed in mg GAE/gEx (y=0.0103x + 0.017; R^2 =0.9991) and the content of total flavonoids, expressed in mgQE/gEx (y = 2.5532x + 0.0285; R^2 = 0.9975) of the hydroethanolic extract. This table shows the contents of phenolic compounds in the hydroethanolic extract of *S. latifolius* leaves. The values are 85.97 mgGAE/gEx for total phenols, 13.26 mgQE/gEx for total flavonoids and 9.19 mgEL/gEx for condensed tannins.

Table 3: Phenolic compound contents of *Sarcocephalus latifolius* leaves

Extract	Total phenols mg GAE/gEx	Total flavonoids mgQE/g Ex	Total tannins (mgEL/gEx)
Hydroethanolic	85.97	13.269	9.192

Antiradical activity

Figure 31 shows the curve of the percentage of trapping of the DPPH· radical as a function of the concentrations of the hydroethanolic extract of *Sarcocephalus latifolius* leaves. A progressive increase in the trapping percentage with the increase in the concentration of the hydroethanolic extract of *Sarcocephalus latifolius* was noted. This curve was used to determine the concentration of the extract making it possible to trap 50% of the DPPH° free radical (IC_{50}). This concentration is 6.6 mg/mL for the hydroethanolic extract of *Sarcocephalus latifolius leaves.* At the level of the aqueous extract (0.299 mg/mL) and the ethanolic extract of *Sarcocephalus latifolius* leaves collected in Benin, Chabi-Sika *et al* [30] noted a more pronounced activity than the hydroethananolic extract. Osama *et al* [31] noted a very low anti-radical activity of the aqueous leaf extract of *Sarcocephalus latifolius* from Sudan (2.015 mg/mL). The interesting anti-radical activity noted in the hydroethanolic extract of *Sarcocephalus latifolius* leaves could be explained by its high phenolic compound content.

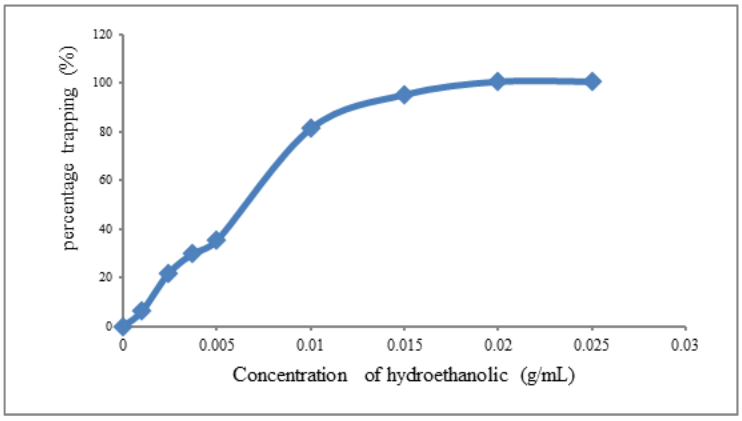

Figure 1: Percentage of trapping of the DPPH radical as a function of the concentrations of the hydroethanolic extract of the leaves of Sarcocephalus latifolius

Inhibition diameter of the hydroethanolic extract of *Sarcocephalus latifolius* leaves on some microbial strains

Table 4 presents the inhibition diameters of the hydroethanolic extract of *Sarcocephalus latifolius* leaves, ciprofloxacin and amoxicillin with the 16 microbial strains tested. With the exception of the strains of *K. pneumoniae* from vaginal samples and *Staphylococcus aureus* (ATCC 29213) which are insensitive to the hydroethanolic extract of *Sarcocephalus latifolius* leaves, the remaining fourteen microbial strains were inhibited by this extract. The hydroethanolic extract of *Sarcocephalus latifolius* leaves inhibited 87.5% of the strains tested while ciprofloxacin and amoxicillin which are synthetic products inhibited respectively 75% and 25% of the strains tested. Our results are in agreement with previous work on the inhibition of microbial strains [32]. On the other hand, the results of the work of Anowi *et al*. [33] showed that the hydroethanolic extract of *Sarcocephalus latifolius* leaves inhibited *Staphylococcus aureus* strains, which was not in our case.

Table 4: Inhibition diameters of *Sarcocephalus latifolius* extract of microbial strains

Microbial strains	Origins	*S. latifolius* ID (mm)	Ciprofloxacin ID (mm)	Amoxicillin ID (mm)
	VS	-	41.5±0.5	19.5±0.5
K. pneumoniae	SP	19.0±1.0	38.0±0.0	16.0±10
	UR	18.0±1.0	36.0±0.5	-
	VS	15.0±0.0	18.5±0.5	-
S. aureus	SP	25.0±0.0	18.5±1.5	-
	UR	19.0±1.0	38.0±0.0	-
	VS	19.5±0.5	41.5±3.5	13.0±0.0
E. coli	SP	16.0±0.0	13.5±0.5	-
	UR	21.5±0.5	14.5±0.5	-
	VS	18.0±0.0	-	-
Streptococcus D	SP	21.0±1.0	40.0±0,0	13.5±1.5
	UR	19.0±1.0	40.0±0.0	-
C. albicans	MHMR	18.0±0.0	-	-
E. coli	ATCC 25922	20.5±1.5	-	-
S. aureus	ATCC 29213	-	38.0±0.0	-
S. pneumoniae	ATCC 49619	20.5±1.5	-	-

Legends: VS: vaginal swab; SP: sperm; UR: urine; *K. pneumoniae: Klebsiella pneumoniae; C. albicans: Candida albicans; E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; S. pneumoniae: Streptococcus pneumoniae;* ID: Inhibition diameters

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the hydroethanolic extract of *Sarcocephalus latifolius* on the strains studied

Table 5 presents the MICs and MBCs of the hydroethanolic extract of *Sarcocephalus latifolius* leaves and synthetic antibiotics (amoxicillin and ciprofloxacin).

Table 5: Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (MBC) of the hydroethanolic extract of *S. latifolius*

Souches		*S. latifolius*		Amoxicilline		Ciprofloxacine	
	Origine	CMI (mg/mL)	CMB (mg/mL)	CMI (mg/mL)	CMB (mg/mL)	CMI (mg/mL)	CMB (mg/mL)
	VS	-	-	10.00	-	0.63	2.50
K. pneumoniae	SP	1.56	6.25	5.00	20.00	0.63	2.50
	UR	1.56	6.25	-	-	1.25	5.00
	VS	6.25	25.00	-	-	0.16	0.63
S. aureus	SP	0.39	1.56	-	-	5.00	20.00
	UR	3.125	12.50	-	-	2.5	10.00
	VS	0.78	3.13	10.00	-	1.25	5.00
E. coli	SP	0.39	1.56	-	-	0.16	0.31
	UR	3.25	12.5	-	-	0.16	0.63
	VS	1.56	6.25	-	-	-	-
Streptococcus D	SP	1.56	6.25	10.00	-	1.25	5.00
	UR	1.56	6.25	-	-	2.50	10.00
C. albicans	MHMR	6.25	25.00	-	-	-	-
E. coli	ATCC 25922	-	-	10.00	-	2.50	10.00
S. aureus	ATCC 29213	0.78	3.13	-	-	-	-
S. pneumoniae	ATCC 49619	6.25	25.00	-	-	0.63	2.50

Legends: VS: vaginal swab ; SP: sperm ; UR: urine; *K. pneumoniae: Klebsiella pneumoniae; C. albicans : Candida albicans; E. coli: Escherichia coli ; S. aureus : Staphylococcus aureus ; S. pneumoniae : Streptococcus pneumoniae*

The hydroethanolic extract of *Sarcocephalus latifolius* leaves inhibited 87.5% of the strains tested, with MICs between 0.39 mg/mL and 6.25 and CMBs between 1.56 mg/mL and 25 mg/mL. It should be noted that strains of *E. coli* (ATCC 25922) and *K. pneumoniae* (vaginal sample) are insensitive to the hydroethanolic extract of *Sarcocephalus latifolius* leaves. The MICs of ciprofloxacin range from 0.125 to 5 mg/mL while the MBCs range from 0.31 to 20 mg/mL. Amoxicillin inhibited only strains of *K. pneumoniae* (vaginal swab), *E. coli* (vaginal swab), *Streptococcus D* (sperm), and E. coli (ATCC 25922) at an inhibitory concentration of 10 mg/mL. On the other hand, it showed bactericidal activity against the strain of *K. pneumoniae* (sperm) at a concentration of 20 mg/mL. The results of this study are in agreement with the work Okwori *et al* [32], Anowi et al [33] which showed that the hydroethanolic extract of *S. latifolius* leaves proved an interesting bactericidal activity against several microbial strains. The antimicrobial activity of the leaf extract of *S. latifolius* can be explained by the presence in the leaves of this plant of metabolites such as tannins, flavonoids, anthocyanins, leuco-anthocyanin saponosides, reducing compounds, mucilages, coumarins, anthraquinones, sterols and triterpenes [29], [34],[34].

6. Conclusion

Medicinal plants remain the primary choice of the population for primary healthcare, not only due to the inaccessibility resulting from the high cost of synthetic products but also due to therapeutic failures caused by the resistance of certain microorganisms. This study aims to valorize *Sarcocephalus latifolius*, a plant used in traditional medicine in Benin to treat urinary tract infections, by determining its secondary metabolites and evaluating its antiradical and antimicrobial activities. According to the results obtained, the leaves of *Sarcocephalus latifolius* contain tannins, flavonoids, anthocyanins, reducing compounds, leuco-anthocyanins, coumarins, saponins, mucilages, and sterols and terpenes. The hydroethanolic extract of *Sarcocephalus latifolius* leaves showed more interesting antimicrobial activity than ciprofloxacin and amoxicillin, which are synthetic products. The diversity of secondary metabolites in Sarcocephalus latifolius leaves and the compelling antimicrobial activity of the hydroethanolic extract of its leaves justify the use of this plant in traditional medicine for treating urinary tract infections.

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