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Comparative *in vitro* antimicrobial effect of *Sarcocephalus latifolius* (Sm.) E. A. Bruce leaves and roots on foodborne pathogens

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***Sarcocephalus latifolius* (Sm.) E. A. Bruce is a plant used traditionally to treat a wide range of infectious diseases, including foodborne ones. This study aimed to compare its roots and leaves extract antibacterial effects *in vitro*. Thus, these organs were collected, dried and powdered for the extractions and phytochemical screening. Four extracts (water, ethyl-acetate, methanol and ethanol) were tested on ten references strains (RS) and nine non references strains of *Staphylococcus* isolated from meat strains (MS) using disk method. Minimal inhibitory (MIC) and bactericidal concentrations (MBC) were determined respectively by macrodilution method and by solid culture medium. The results showed that the MS seem more susceptible than RS. The lowest activity was shown by roots hydro-ethanolic extract on *Micrococcus luteus*, whereas the highest was obtained with leaves ethanolic extract on *Staphylococcus cohini*. The MIC ranges from 0.312 to 25 mg/ml for the MS and from 6.25 to 12.50 mg/ml for the RS. About MBC, they vary from 12.50 to 50 mg/ml for all susceptible tested microorganisms. *S. latifolius* extracts have an inhibitory effect on both strains such as *Staphylococcus xylosum*, *Escherichia coli* O157, *Staphylococcus sciuri*, *Staphylococcus aureus*, *M. luteus*, *Candida albicans*, *S. cohinii*, *Proteus mirabilis* and bactericidal effect on the three last ones. These results support the traditional use of *S. latifolius* in infectious diseases control; furthermore, its leaves seem more effective than the roots. These findings may serve as a starting point for the development of a new drug for the control of this kind of diseases and could sustain the species use.**

Key words: Pathogens, medicinal plant, *Sarcocephalus latifolius*, phytochemical screening, metabolites.

INTRODUCTION

Plants are used for centuries for health care taking. Several studies have been conducted during the last decades, concerning their chemical composition and antimicrobial activities which enhanced their use. They

are a source of manufactured drugs in developed countries and are directly used as infusion or decoction in developing ones. Infectious or parasitic diseases (gastro-intestinal ailments, hepatitis, tetanus etc.) are serious

public health care problems worldwide (Murray and Lopez, 1997) causing seventeen million deaths, from which, the half is in African countries (WHO, 2003). In 2013, 80.2 million diseases cases were foodborne with an increase of 51.1% since 1990 (Murray et al., 2015). They impede people's health and have a negative impact on the human development index (Petri et al., 2008). These mainly are observed in areas characterized by poor hygiene, lack of drinkable water and health care access, and their rate is expected to increase with current global warming (Altizer et al., 2013; Ong et al., 2018). Worldwide, 22.9% of Disability Adjusted Life Year (DALYs) was caused by infectious and parasitic diseases, but this proportion ranged from 2.7% in formerly socialist economies of Europe to 42.5% in sub-Saharan Africa (Murray and Lopez, 1997). Among those illnesses, diarrheal and foodborne diseases have the highest rate (Kirk et al., 2015). Even in the United States, each year approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths are reported (Mead et al., 1999), their highest prevalence concern the African region (10.3, 0.01 and 1%) followed by the South East Asian region (8.07, 0.01 and 0.47%) considering the illnesses cases, deaths and DALYs respectively (Kirk et al., 2015). Foodborne diseases, including both foodborne intoxications and foodborne infections, are terms applied to illnesses acquired by consumption of contaminated food; they are frequently and inaccurately referred to as food poisoning (Chin, 2000).

In Benin like elsewhere in Africa, infectious or parasitic diseases are the primary public health care problem after diseases of the blood or blood-forming organs and specific disorders involving the immune system (malaria, hemorrhagic fever, anemia, icterus, etc.) as Ahoyo et al., (2019). Moreover, these diseases were currently categorized as neglected tropical diseases (Herricks et al., 2017) and need urgent actions to fight them. In this frame, due to inaccessibility to modern medicine, mainly in rural areas, and current pathogens resistance to those medicines, the best option remains to find a local and natural solution to mitigate those health care problems. *Khaya senegalensis* (Desr.) A. Juss., *Sarcocephalus latifolius* and *Vitellaria paradoxa* C. F. Gaertn. were identified as the main woody species involved in their traditional treatment (Ahoyo et al., 2019). However, *S. latifolius* was the most used due to its medicinal properties regarding stomach disorders and foodborne diseases (Badiaga, 2011; Yinusa et al., 2012; Kaboré et al., 2014).

A native of the African country, *S. latifolius* also called African peach, is a Sudano-Guinean species primarily found in west intertropical Africa. Having 5 to 9 m of

height, it is a shrub of about 30 cm of diameter. It has flexible branches, fibrous bark, yellow or white timber, simples and opposite leaves and yellow roots. The leaves remain green through the year and flowers arise from January to May.

Moreover, it is involved in the treatment of other diseases such as diabetes (Iwueke et al., 2010; Karou et al., 2011), AIDS (Lamorde et al., 2010), malaria (Idowu et al., 2010; Adomou et al., 2017; Ahoyo et al., 2018), menstrual disorders (Deleke Koko et al., 2011) and for infant caretaking (Kaboré et al., 2014). It is mainly used by African women in Kinshasa (DR Congo) for their intimate hygiene (Ngbolua et al., 2014) and has also veterinary properties such as treatment of bovine Pasteurellosis (Dassou et al., 2014). It is also used as a condiment in Burkina-Faso (Kaboré et al., 2014).

Within medicinal plants trading, *S. latifolius* roots are the most common selling in Benin (Quiroz et al., 2014). In Cameroon, its roots are used for fever, convulsion, headache, inflammatory and neuropathic treatments, whereas leaves are involved in the treatment of nervous system disorders like epilepsy or anxiety (Taïwe et al., 2014). Its roots are the most used plant parts in medicine (Kaboré et al., 2014).

The high use of its roots against diarrheal infectious is linked to their coumarins and polyphenolic compounds which possess anti-inflammatory, antimicrobial and anticoagulant properties (Badiaga, 2011). Several studies are mainly focused on its roots by assessing its efficacy *in vitro* [antileishmanial (Ahua et al., 2007), against Herpes simplex virus type 1 and African swine fever virus (Silva et al., 1997)] and *in vivo* [antidiarrheal (Owolabi et al., 2010), antimalarial, against nociception, inflammation, and pyrexia (Abbah et al., 2010), hepatoprotective (Yesufu et al., 2010)].

The roots utilization could jeopardize the species survival than the leaves (Kaboré et al., 2014). Hence, it is necessary to compare its leaves antimicrobial activity to the roots ones. The leaves will be advocated whether the two parts have at least the same effects; moreover, the leaves can contain a high number of secondary metabolites and could be more effective in disease treatment (Nacoulma-Ouédraogo, 1996). In this frame, this study aims to assess and compare the *in vitro* antimicrobial activity between *S. latifolius* leaves and roots to foodborne pathogens.

MATERIALS AND METHODS

Tested microorganisms

The nineteen microorganisms that were tested are composed of:

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- i. Ten (10) reference strains with five Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *S. epidermidis* T22695, *Micrococcus luteus* ATCC 10240, *Streptococcus oralis* and *Enterococcus faecalis* ATCC 29212); four Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* A24974, *Proteus vulgaris* A 25015 and *Escherichia coli* ATCC 25922) and one yeast (*Candida albicans* MHMR);
- ii. Six (06) *Staphylococcus* species (*S. sciuri*, *S. aureus*, *S. simulans*, *S. xylosum*, *S. cohnii* and *S. haemolyticus*) isolated from three different meat products in Ivory Coast by Attien *et al.* (2011) and stored in the Laboratory of Biology and Molecular Typing in Microbiology (University of Abomey-Calavi, Benin);
- iii. Two (02) *Salmonella enterica* serovars (*Salmonella typhi* and *S. paratyphi*) isolated from meat and
- iv. Two (02) clinical strains of *E. coli* isolated from diarrheal infections.

The non-reference isolates were obtained from the Laboratory of Biology and Molecular Typing in Microbiology of the University of Abomey-Calavi of Benin.

Plant material collection

The leaves and roots of *S. latifolius* were collected in the Monts Kouffé forest in Center of Benin. Voucher specimens (AA 6739 / HNB) were collected and deposited in the National Herbarium of Benin. These plant organs were cut into pieces, dried between 25 and 30°C at the laboratory for 15 days and then ground to powdered-form which was then kept in an air-tight plastic bag until use. The powder was used for extraction with five solvents (ethanol, hydro-ethanol, methanol, hexane and ethyl acetate).

Hydro-ethanolic and methanolic extraction

An adaptation of Guédé-Guina *et al.* (1995) protocol was used. Briefly, 50 g of powder has been macerated in 500 ml of solvents (distilled water-ethanol (v/v) or methanol) on electronic agitator for 48 h at ambient temperature (20°C). The obtained product was filtered twice with hydrophilic cotton and once on Whatman No 1 paper. This filtrate was dried at 45°C for total hydro-ethanolic and methanolic extracts obtaining.

Successive extraction with ethanol, hexane and ethyl-acetate

The extracts were obtained according to the method described by N'Guessan *et al.* (2007). One hundred (100) g of powder was macerated in 1000 ml of ethanol 96° under agitator for 48 h at ambient temperature. The homogenate was filtered thrice on hydrophilic cotton and once on Whatman No 1. The 1/5 of the obtained filtrate was evaporated at 40°C to get the ethanolic extract. To 4/5 fraction, was added 50 ml of hexane. After agitation, the mixture was transferred within decanter ampoule. The upper phase was reaped and evaporated to get the hexanic extract. After that, 50 ml of ethyl acetate and 50 ml of distilled water were added to the lower phase and shook for 20 min in a decanter ampoule. Finally, the upper phase was reaped and evaporated for ethyl acetate extract obtaining.

Phytochemical screening

The qualitative phytochemical screening was performed based on coloring or precipitation reactions. It is made directly on the powder of the *S. latifolius* organ according to Ali *et al.* (1998).

Antimicrobial activity assessment

Sensibility tests

The antimicrobial activity of the extracts was evaluated using the disk diffusion method described by Bauer *et al.* (1966). Indeed, 1 ml of bacterial culture (adjusted to 0.5 McFarland standards) was spread on a Petri dish containing Mueller-Hinton agar (Bio-Rad, France) (SFM, 2008). Two to four sterile disks (5 mm) are deposited in the Petri dish previously flooded with bacterial culture under aseptic conditions. These disks were inoculated with 30 µl of tested extract (50 mg/ml). The disk was then kept at room temperature 15-30 min before being incubated at 37°C for 24 h (Adesokan *et al.*, 2007) and 48 h. The inhibition diameters were measured using a scale after incubation times of 24 and 48 h. The experience was repeated twice for each extract.

Determination of minimum inhibitory concentration (MIC)

The method of macrodilution with visual observation previously used by Dah-Nouvlessounon *et al.* (2015) was used. Firstly, the extracts were diluted in sterilized distilled water to the highest concentration of 50,000 µg/ml and then nine dilutions were performed to obtain the concentrations of 25,000, 12,500, 6,250, 3,125, 1,562.5, 781.25, 390.62, 195.31 and 97.65 µg/ml in screw capped. Then, 1 ml of the bacteria inoculum (10⁶ cfu/ml) was added to 1 ml of the above mentioned concentrations to obtain 2 ml as a final volume. Culture medium without extracts and others without microorganisms were used as a control. Tubes were incubated at 37°C for 18 - 24 h and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth (turbidity).

Determination of minimum bactericidal concentration (MBC)

Referring to the results of MIC test, all tubes showing no microorganism growth were identified. Each tube was inoculated into a Petri dish containing MH agar and incubated at 37°C for 24 h. The lowest concentration of the extract in which the microorganism did not grow on solid medium was considered as the Minimum Bactericidal Concentration (Moroh *et al.*, 2008).

Identification of inhibitory and bactericidal effect of extracts

Bactericidal or inhibitory effect of extract depends on the ratio CMB/CMI (Berche *et al.*, 1991). It is bactericidal when the ratio is comprised between 1 and 2; and inhibitory if it is between 4 and 16.

Data analysis

The extraction yielding was obtained by dividing the anhydrous mass of the extract by plant material used (Harborne, 1998). The used formula is:

$$R = \frac{Me}{Mp} \times 100$$

With R, the yield in percentage; Me, the anhydrous mass of the extract; and Mp, the plant material's mass.

A principal component analysis using the package *FactoMineR* (Le *et al.*, 2008) and *Factoextra* (Kassambara and Mundt, 2017) showed the sensibility pattern of tested microorganisms (10

Table 1. Leaves and roots of *S. latifolius* yield.

Type of extract	Water-ethanolic		Ethanolic		Methanolic		Ethyl acetate	
Organ	Leave	Root	Leave	Root	Leave	Root	Leave	Root
Powder mass (g)	100	100	50	50	25	25	50	50
Extract mass (g)	6.6	6.6	3.4	3.9	3.4	3.2	3	2.5
Yield (%)	6.6	6.6	6.8	7.8	13.6	12.8	6	5

Table 2. Phytochemical compounds of *S. latifolius* organs.

Family	Class	Leave	Root
Nitric compounds	Alkaloids	++	++
	Tannins	+++	+++
	Catechetic tannins	+++	+++
	Gallic tannins	+++	+++
Poly-phenolic compounds	Flavonoid	+++	+++
	Anthocyanin	-	-
	Leuco-anthocyanins	-	-
	Coumarin	++	++
	Quinonics derived	++	++
	Triterpenoids	+++	+++
Terpenic compounds	Steroids	+++	+++
	Cardenolides	-	-
	Cyanogeniques derived	-	-
	Saponosides (IM)	+++	+++
Heterosides or glycosides	Reducing Compounds	+	+
	Free anthracenics	-	-
	O-heterosides	-	-
	O- heterosides at GR	-	-
	C-heterosides	-	++
	Mucilage's	-	++

+/- indicates presence/absence.

references and 9 alimetal strains) to leaves and roots extract (ethanolic, acetic, hydro-ethanolic and methanolic) of *S. latifolius*. Student *t* test and student-Newmann-Keuls were used for specifying the efficacy gradient of those extracts on more susceptible microorganisms. The analyses were performed using R software, version 3.4.3 (R Core Team, 2017).

RESULTS

Extraction yield

The extraction yielding by each solvent was shown in Table 1. It varied following the kind of extracts and plant organs used. The methanolic extract has the highest yield (13.6% for the leaves and 12.8% for the roots), whereas the acetic extract has the lowest (5%). The leaves and the roots yield followed the same trend.

Phytochemical profiling

Various primary compounds are present in leaves and roots of *S. latifolius*. Among researched compounds, Anthocyanins and Leuco-anthocyanins were absent within polyphenolics class whereas derived cardenolides and cyanogenic were absent among Terpenics ones. Among heterosides, only the roots contain C-heterosides and mucilages as presented in Table 2.

Antimicrobial activity

Sensibility of tested microorganisms to leaves and roots extracts of *S. latifolius*

The sensibility of tested microorganisms to plant extracts

Table 3. Antimicrobial activity of leaves and roots of *S. latifolius* on tested reference and alimental strains.

Microorganism	LEE	LME	LHEE	LAE	REE	RME	RHEE	RAE
Reference strains	<i>S. aureus</i>	-	-	-	-	+	+	-
	<i>P. aeruginosa</i>	-	-	-	+	-	+	-
	<i>P. mirabilis</i>	+	-	+	+	-	-	-
	<i>M. luteus</i>	--	-	-	-	+	+	-
	<i>S. epidermidis</i>	+	+	-	+	-	-	+
	<i>P. vulgaris</i>	-	-	-	-	-	-	-
	<i>S. oralis</i>	+	+	-	-	-	-	-
	<i>E. faecalis</i>	-	-	+	+	-	-	-
	<i>E. coli</i>	-	-	-	-	-	-	-
	<i>C. albicans</i>	+	+	+	+	-	-	-
Isolated meat strains	<i>S. sciuri</i>	-	-	+	-	+	+	+
	<i>S. haemolyticus</i>	-	-	-	-	-	+	-
	<i>S. simulans</i>	-	-	-	-	-	-	-
	<i>S. xyloxy</i>	-	+	-	-	-	-	+
	<i>S. cohnii</i>	+	-	+	+	-	-	-
	<i>E. coli</i> O157	-	+	+	+	-	-	+
	<i>E. coli</i>	-	-	-	-	-	-	-
	<i>S. Paratyphy</i>	-	-	-	-	-	-	-
	<i>S. Typhy</i>	-	-	-	-	-	-	-

+: inhibition; -: not inhibition; LEE: Leaves ethanolic extract; LHEE: Leaves hydro-ethanolic extract; LME: Leaves methanolic extract; LAE: Leaves acetic extract; REE: Roots ethanolic extract; RHEE: Roots hydro-ethanolic extract; RME: Root methanolic extract; RAE: Roots acetic extract.

was observed at 50 mg/ml of extracts (Table 3). At 20 mg/ml, the extracts did not produce any effect on those microorganisms.

The isolates of meat origin seemed to be more susceptible than reference ones. In fact, the lowest activity was shown by roots hydro-ethanolic extract (RHE) on *M. luteus* (reference strains), whereas the highest was observed by leaves ethanolic extract (LEE) on *S. cohnii* as illustrated in Figure 1.

The first two axes of the principal component analysis (PCA) performed on the matrix of the inhibition diameters, explained 61% of the total variation among the microorganism susceptibility to extracts and revealed five groups as shown in Figure 2. About 68% (13) of tested microorganisms were sensible to plant extracts. The groups are:

- i. Group G0: *P. aeruginosa*, *P. mirabilis*, *S. epidermi*, *E. faecalis*, *S. haemolyticus*, *S. sylosus* and *S. cohnii* with a mean sensibility to all tested extracts.
- ii. Group G1: *S. oralis* is susceptible to leaves ethanolic extract (LEE).
- iii. Group G2: *S. aureus*, *M. luteus* and *S. sciuri* are very susceptible to roots methanolic extracts (RME).
- iv. Group G3: leaves hydro-ethanolic (LHEE) and acetic extract (LAE) are efficacious on *C. albicans*.
- v. Group G4: *E. coli* O157 is equally (p-value = 0.2774) susceptible to leaves methanolic (LME) and roots acetic

extracts (RAE).

Minimum inhibitory (MIC) and bactericidal concentration (MBC)

The plants' extract showed various MIC and MBC values according to tested microorganisms. The MIC obtained for both reference and meat isolates were presented in Table 4 whereas the MBC were presented in Table 5.

Bactericidal and inhibitory extracts

Based on CMB/CMI reports, ethanolic extract of leaves had a bactericidal effect on *C. albicans* and *P. mirabilis*. The same effect was observed about hydroethanolic extracts of leaves on *S. cohnii*. The remaining extracts had just inhibitory effects on the other tested microorganisms as presented in Table 6.

DISCUSSION

Several parameters affect the extraction procedure such as the chemical form of investigated compounds, the extraction method, the size of sampled particles, the used plant parts, the solvent polarity, the drying conditions and

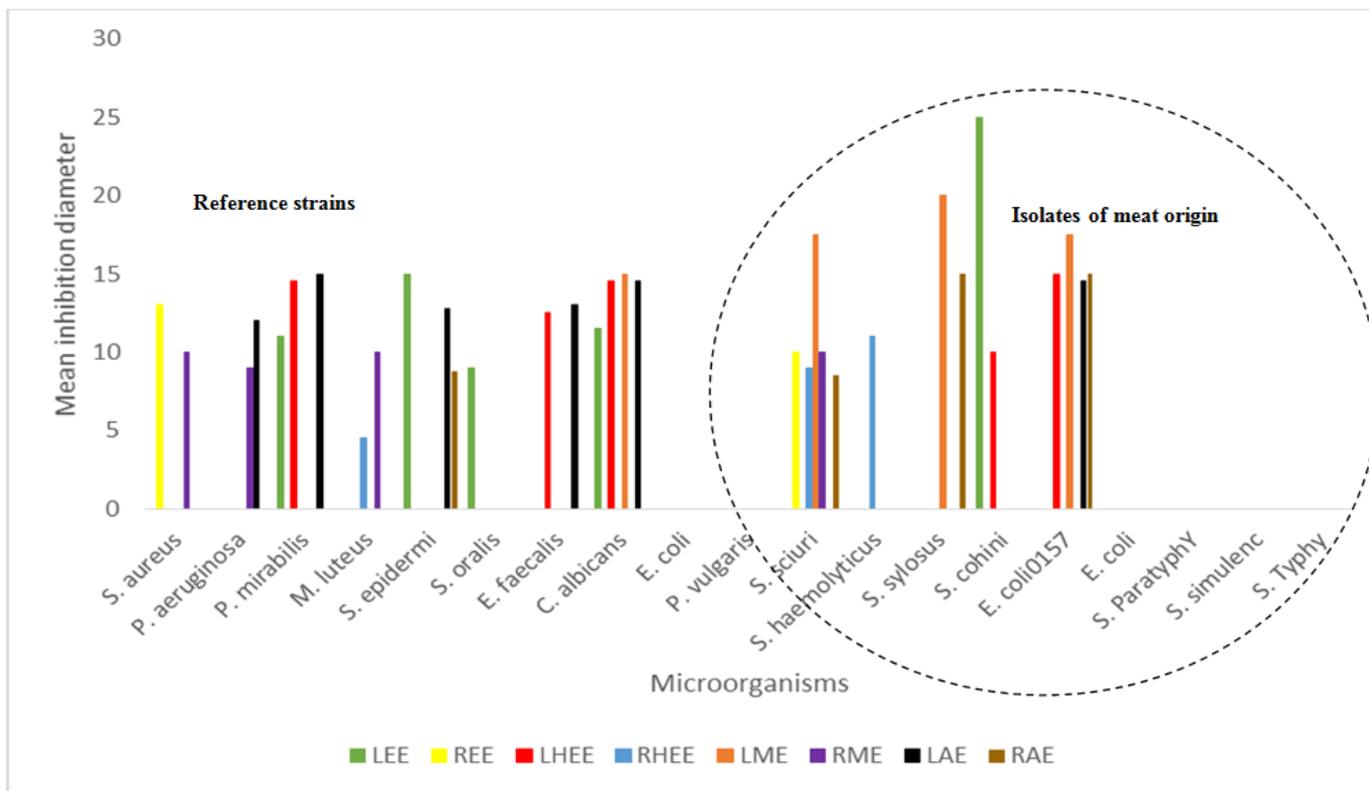


Figure 1. Growth inhibition diameter of tested microorganisms induced by *S. latifolius* leaves and roots extracts.

the extraction time (Herzi, 2013; Mohammadi, 2013; Nazck and Shahidi, 2004). The extracts yield is overall very underneath than 50%; it can be due to one or more of the above reasons. Indeed, the plant materials also contain some secondary metabolites other than those revealed by used solvents and methods.

The methanolic extract produced the highest yield, whereas the lowest was obtained with the ethyl acetate ones. It could be because of the extraction capacity of a solvent depends on its polarity and affinity for phyto-molecules (Dah-

Nouvlessounon et al., 2015). Furthermore, the majority of natural's products seem more soluble in methanol than other more polar solvents (Telli et al., 2010). In this way, Telli et al. (2010) found this solvent, as the most efficient for polyphenols extraction from date fruits. Unlikely, Ibanez et al. (2003) showed that the extraction yield was positively correlated with solvent polarity.

Even no polar solvent (hexane for example) showed sometimes better extraction yield (Herzi, 2013). To improve the extraction yield, link to the tested metabolites, a selective extraction using a

solvent of an appropriate polarity driving by the principle of *like dissolves like* is advocated. Thus, nonpolar solvents are used to solubilize mostly lipophilic compounds (alkanes, fatty acids, pigments, waxes, sterols, some terpenoids, alkaloids and coumarins). Medium-polarity solvents are used to extract compounds of intermediate polarity (some alkaloids, flavonoids), while more polar ones are used for more polar compounds (flavonoid glycosides, tannins, some alkaloids). Water is not used often as an initial extractant, even if the aim is to extract water-

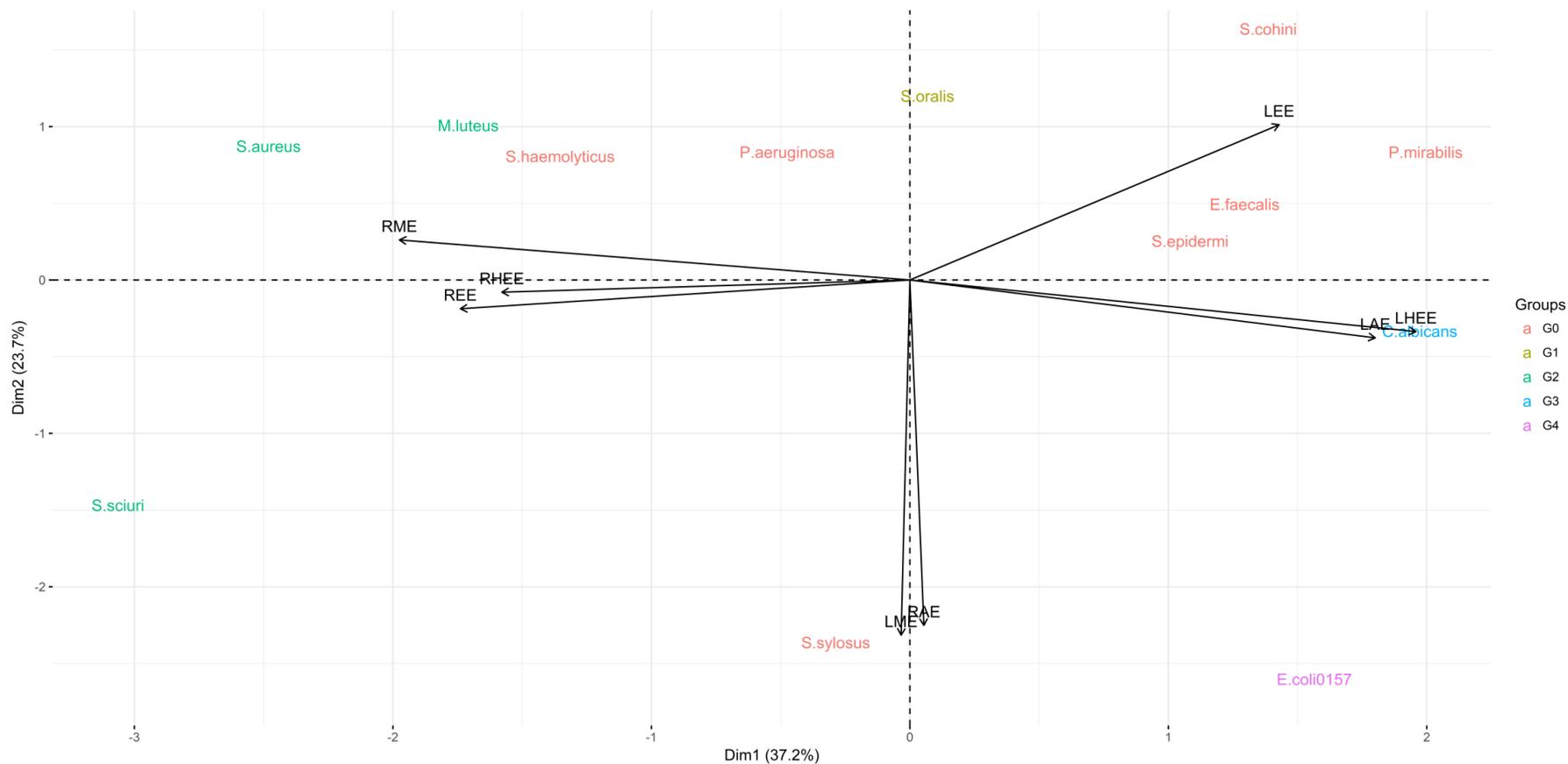


Figure 2. Pattern of tested microorganism's sensibility to *S. latifolius* extracts.

soluble plant constituents (glycosides, quaternary alkaloids, tannins) (Sarker et al., 2006). Further supercritical fluid extraction studies may undertake to improve the extraction yield regarding their particular faculties for natural products extraction from plants (Castioni et al., 1995).

Concerning observed variations between the yields of leaves and roots extractions, it may be due to the chemical composition of those plant

organs and the physiological status of the harvested plant species. The yield was better with leaves extracts than roots. It is well known that the quantity of secondary metabolites has a variable distribution through different plant organs (Figueiredo et al., 2008). Moreover, leaves as the plant photosynthesis center, hold the greatest rate of those secondary metabolites.

Leaves and roots of *S. latifolius* contain the

major kinds of secondary metabolites such as alkaloids, tannins, flavonoids, terpenes and saponins. The identified secondary metabolites within those organs are well known for their biological activities (antiviral, antibacterial, anti-inflammatory, etc). Moreover, the total absence of cyanogenic heterosis confirmed the non-toxic effect of this species and hence, allows its great use in folk medicine like showed by other studies

Table 4. Minimum inhibitory concentration (mg/ml) of plant extracts.

Microorganism	LEE	LME	LHEE	LAE	REE	RME	RHEE	RAE	
Reference strains	<i>S. aureus</i>	0	0	0	0	6.25	12.50	0	0
	<i>P. aeruginosa</i>	0	0	0	6.25	0	12.50	0	0
	<i>P. mirabilis</i>	12.50	0	6.25	6.25	0	0	0	0
	<i>M. luteus</i>	0	0	0	0	25	6.25	0	0
	<i>S. epidermidis</i>	12.50	12.50	0	6.25	0	0	0	6.25
	<i>P. vulgaris</i>	0	0	0	0	0	0	0	0
	<i>S. oralis</i>	50	25	0	0	0	0	0	0
	<i>E. faecalis</i>	0	0	12.50	12.5	0	0	0	0
	<i>E. coli</i>	0	0	0	0	0	0	0	0
	<i>C. albicans</i>	6.25	6.25	12.50	6.25	0	0	0	0
Isolated meat strains	<i>S. sciuri</i>	0	0	3.12	0	6.25	6.25	12.50	25
	<i>S. haemoliticus</i>	0	0	0	0	0	0	12.50	0
	<i>S. simulans</i>	0	0	0	0	0	0	0	0
	<i>S. xylosus</i>	0	3.12	0	0	0	0	0	6.25
	<i>S. cohnii</i>	3.12	0	12.50	12.50	0	0	0	0
	<i>E. coli</i> O157	0	6.25	3.12	6.25	0	0	0	6.25
	<i>E. coli</i>	0	0	0	0	0	0	0	0
	<i>S. Paratyphy</i>	0	0	0	0	0	0	0	0
	<i>S. Typhy</i>	0	0	0	0	0	0	0	0

LEE: Leaves ethanolic extract; LHEE: Leaves hydro-ethanolic extract; LME: Leaves methanolic extract; LAE: Leaves acetic extract; REE: Roots ethanolic extract; RHEE: Roots hydro-ethanolic extract; RME: Root methanolic extract; RAE: Roots acetic extract.

(Ahojo et al., 2018; Kaboré et al., 1995). Also, tannin's antiseptic, antifungal and antibacterial effects were shown by Mesia et al. (2005). Their presence in both leaves and roots explain this species prescription as antidiarrheal or gastrointestinal disorders regulator.

Deleke Koko et al. (2011), found Anthocyanins and C-heterosides within leaves and roots of *S. latifolius* whereas, those compounds were absent within the present study findings. Also, Alkaloids, derived quinones, gallic tannins, steroids and triterpenoids revealed in the present phytochemical screening were not found by Deleke Koko et al. (2011). These changes may be due to the variation of environmental conditions of the plants' collection areas which are under different climatic characteristics. They collected their plants at Pendjari, Sudanian zone of Benin, while there have been collected in the Sudano-Guinean zone of Benin for this study. Even Badiaga (2011) found in Mali that climate variation did not influence the plant metabolites production, their effect seems currently irrefutable.

Ethanolic, hydro-ethanolic, methanolic and ethyl acetate extracts of leaves and roots of *S. latifolius* inhibited at 50 mg/ml, 68.42% of the growth of the tested microorganisms. Although, hydro-ethanolic extracts seem more efficacious. The extracts were efficacious on *P. mirabilis*, *S. cohnii*, *C. albicans*, *M. luteus*, *S. aureus*, *S. sciuri*, *E. coli* O157 and *S. xylosus*. Among the pathogens of greatest concern today, is *E. coli* O157 (Mead et al., 1999).

The foodborne strains seem more susceptible than reference ones. This lack of resistance can be due to some mutations they face in natural environment or within the biological systems (like animal's body) within which they live. In this sense, Anowi et al. (2017) and Okwori et al. (2008) found similar results in antimicrobial activity of *S. latifolius* leaves assessing. Although, *S. Typhi*, not sensible in this study was found to be sensible to this species leaves alcoholic extracts (Sourabie et al., 1995). This difference of sensibility may be due to the difference between the used alcohol concentrations.

Minimum inhibitory and bactericidal concentration varied following kinds of extracts and strains. They inhibited the growth of the majority of tested strains, both Gram – and Gram +. The minimum inhibitory concentration ranged from 0.312 to 25 mg/ml.

Leaves ethanolic extracts had a bactericidal effect on both yeast (*C. albicans*), and Gram – reference strains (*P. mirabilis*), whereas the hydro-ethanolic had the same effect on Gram + foodborne strain (*S. cohnii*). The only difference between those two extracts was about concentration.

Leaves hydro-ethanolic extracts can be obtained by increasing the ethanolic extract volume with distilled water. Likewise, ethanolic extracts of leaves of *S. latifolius* seems efficacious on the majority of bacterial classes independently on their sources. Extract efficacy depends strongly on plants species, the used part, the kind of solvent and also to tested microorganisms. About

Table 5. Minimum Bactericidal Concentration of plants extracts.

Microorganism	LEE	LME	LHEE	LAE	REE	RME	RHEE	RAE	
Reference strains	<i>S. aureus</i>	0	0	0	0	25	50	0	0
	<i>P. aeruginosa</i>	0	0	0	12.50	0	50	0	0
	<i>P. mirabilis</i>	25	0	25		0	0	0	0
	<i>M. luteus</i>	0	0	0	0	50	25	0	0
	<i>S. epidermidis</i>	50	12.50	0	25	0	0	0	25
	<i>S. oralis</i>	50	25	0	0	0	0	0	0
	<i>E. faecalis</i>	0	0	50	50	0	0	0	0
	<i>C. albicans</i>	12.50	12.50	50	25	0	0	0	0
	<i>E. coli</i>	0	0	0	0	0	0	0	0
	<i>P. vulgaris</i>	0	0	0	0	0	0	0	0
Isolated meat strains	<i>S. sciuri</i>	0	0	12.50	0	25	25	50	50
	<i>S. xylosus</i>	0	12.50	0	0	0	0	0	25
	<i>S. cohnii</i>	12.50	0	25	25	0	0	0	0
	<i>E. coli O157</i>	0	25	12.50	25	0	0	0	25
	<i>E. coli</i>	0	0	0	0	0	0	0	0
	<i>S. haemoliticus</i>	0	0	0	0	0	0	-	0
	<i>S. Paratyphy</i>	0	0	0	0	0	0	0	0
	<i>S. simulans</i>	0	0	0	0	0	0	0	0
	<i>S. Typhy</i>	0	0	0	0	0	0	0	0

Legend: LEE: Leaves ethanolic extract; LHEE: Leaves hydro-ethanolic extract; LME: Leaves methanolic extract; LAE: Leaves acetic extract; REE: Roots ethanolic extract; RHEE: Roots hydro-ethanolic extract; RME: Root methanolic extract; RAE: Roots acetic extract.

the solvent, it is advocated to use several reagents with different polarity to get the maximum of secondary metabolites contained within the plant materials. Concerning their efficacy on microorganisms, it depends on the different resistance opposed by the membrane of those microorganisms. Indeed, the action mode of the extracts on this membrane is determinant within its efficacy.

Moreover, leaves extracts had a great inhibition zone on bacterial growth than roots ones. Okwori et al. (2008) also found similar results; these results imply that the leaves of *S. latifolius* could be used instead of its roots. Indeed, several findings through the world established that roots are the most used part for this species, as its most efficacious part (Kaboré et al., 2014; Tabuti et al., 2003). Its overuse constitutes an aggravating factor for their survival (Kaboré et al., 2014) and then compromises seriously its durability. The most direct ecological consequence of non-timber forest products extraction is an alteration of the rates of survival, growth and reproduction of harvested individuals (Ticktin, 2004). The present finding could be used to sustain this species collection.

Furthermore, it would be beneficial to improve the isolation, purification and assess the contents secondary metabolites of this species like Betasitosterol (Yinusa et al., 2015); strictosamide (Abreu and Pereira, 1998;

Candeias et al., 2009); and indol-alkaloids (Nworgu et al., 2010) for antimicrobial activity. Hence, the pure components would be used more safely and efficaciously.

Conclusion

To better evaluate the used species in traditional medicine, this study highlighted *S. latifolius* compounds and their efficacy on some microorganisms. The species extracts deal with tested microorganisms at dose non-depending on their sources and strains. Ethanol was the best solvent for the extraction of this species material in order to assess its pharmacological activities. The results support strongly the use of *S. latifolius* in folk medicine for infectious and parasitic diseases treatment and advocate preferring the leaves instead of its roots in such diseases combating.

For better accuracy of its use, further studies will focus on the fractioning of its brutes' extracts in order to isolate pure efficacious compounds and their suitable dosage. Clinical studies starting with those results can allow new specific drug manufacture to deal with foodborne diseases. Even, as saying, prevention is better than cure, prevention by avoidance of undercooked meat or seafood, unpasteurized milk or soft cheese, could also serve as a key to the control of foodborne diseases.

Table 6. Inhibitory and bactericidal effects of *S. latifolius* on tested microorganisms.

Extract	Strain	CMB/CMI
LAE	<i>C. albicans</i>	4
	<i>P. mirabilis</i>	-
LME	<i>S. xylosus</i>	4
	<i>E. coli</i> O157	4
LEE	<i>C. albicans</i>	2
	<i>P. mirabilis</i>	2
	<i>S. cohnii</i>	4
LHEE	<i>C. albicans</i>	4
	<i>P. mirabilis</i>	4
	<i>S. cohnii</i>	2
RAE	<i>S. xylosus</i>	4
	<i>E. coli</i> O157	4
RHEE	<i>M. luteus</i>	-
	<i>S. sciuri</i>	4
REE	<i>S. aureus</i>	4
	<i>S. sciuri</i>	4
RME	<i>S. aureus</i>	4
	<i>M. luteus</i>	4
	<i>S. sciuri</i>	4

Legend: LEE: Leaves ethanolic extract; LHEE: Leaves hydro-ethanolic extract; LME: Leaves methanolic extract; LAE: Leaves acetic extract; REE: Roots ethanolic extract; RHEE: Roots hydro-ethanolic extract; RME: Root methanolic extract; RAE: Roots acetic extract.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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