



Antimicrobial Activities of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae) Fruit Extracts on Six Standard Microorganisms and Their Clinical Counterpart

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Abstract

Syzygium aromaticum is used in combination with other plants as an alcoholic infusion by traditional practitioners to treat infections. It has been selected for evaluation for its antimicrobial properties to justify its use in traditional pharmacopoeia. The fruits were used as plant material while the microbial germs consisted of six reference strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexneri* ATCC 12022, *Candida albicans* ATCC 35659 and their clinical counterparts. The extracts were obtained by aqueous decoction, hydroethanolic and ethanolic macerations. The phytochemical screening was performed by chemical staining tests. The antibiotic susceptibility test was performed using the well diffusion method and the MIC and MBC or MFC were determined using the 96-well microplate dilution method. The results showed that 66.67% of the strains tested were sensitive to the aqueous extract with inhibition diameters ranging from 15 to 21 mm and MIC and MCB or MFC between 0.0976 - 0.3906 mg/mL and 0.1953 - 0.7812 mg/mL respectively, thus determining bacteriostatic activity. 100% of the germs tested were sensitive to hydroethanolic and ethanolic extracts. The inhibition diameters range from 12 - 28 mm for hydroethanolic extract with MIC and MBC or MFC ranging from 0.0488 - 0.3906 mm and 0.0488 - 0.7812 mm respectively. The ethanolic extract gave inhibition diameters of 12 - 26 mm; MIC and MBC or MFC ranging from 0.0976 - 0.7812

mm. Hydroethanolic extract gave three (3) bactericidal/fungicidal activities compared to four (4) as for ethanolic extract. These results prove the use of *S. aromaticum* among traditional recipes for treating infections in the pharmacopoeia but further studies remain important to produce traditionally improved drugs.

Subject Areas

Pharmacology

Keywords

Syzygium aromaticum, Phytochemical, Antibacterial, Antifungal

1. Introduction

The traditional use of plants for health care by the peoples of the world dates back to ancient times. Nowadays, plants continue to be used as a cure for various diseases despite advances in modern medicine; this is due to the perpetual phenomena of resistance of microbial agents to conventional antibiotics and their side effects [1]. This situation not only leads the laboratories to look to the plants for the research of new active ingredients but also the populations to be attached to the medicinal plants which indeed are effective, having less side effects and accessible with affordable costs [1]. Moreover, the rationale for traditional medicine, which uses plants for health care, comes from the fact that in developing countries most people have low incoming power and access to health services is limited in some areas.. But these plants must be studied in order to establish the scientific evidence of their uses. For example, an ethnobotanical survey of sellers of alcoholic herbal infusions in Lomé city identified several plants which supposedly treat urinary, genital, throat and pulmonary infections. This work consisted on the one hand to map and then to identify all the plants used for the infused recipes and on the other hand to evaluate the antibiotic activity of some plants against the germs involved in the aforementioned pathologies. Among the listed plants is *Syzygium aromaticum* commonly known as clove. It is an ever-green aromatic plant 10 to 20 m tall belonging to the Myrtaceae family, native to the Maluku Islands of Eastern Indonesia [2] [3]. It is used in traditional medicine to deal with nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distention and gastrointestinal spasms, pain, to cause uterine contraction and stimulate nerves [4] [5] [6]. Scientific research has shown that cloves have antimutagenic [7], antiulcer [8], antithrombotic, antioxidant [9] [10], anti-inflammatory [11], antiparasitic, antiseptic, antiviral and antimicrobial properties [3] [12]. With regard to antimicrobial studies, many germs are still to be tested in order to identify the spectrum of activity of the plant. It is in this perspective that this research aims to determine the susceptibility of reference germs and their clinical counterpart to *S. aromaticum* spices extracts.

2. Material and Methods

The material consists of fruits (spices) of *S. aromaticum*, harvested in Tabligbo, Togo. The plant has been identified in the Laboratory of Botany and Plant Ecology of the Faculty of Sciences (FDS) of the University of Lomé (UL). A voucher specimen was deposited in the herbarium of this laboratory under the number TG15323. Then the fruits were washed and dried under air conditioning ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in the laboratory of Physiology Pharmacology of the FDS. They were finally reduced to powder with a mortar for manual use, kept in sealed boxes protected from light for extraction and stored at room temperature.

The microorganisms consist of the reference strains and their clinical counterpart: *Escherichia coli* ATCC 25922, *Escherichia coli*, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus*, *Salmonella typhimurium* ATCC 14028, *Salmonella typhi*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa*, *Shigella flexneri* ATCC 12022, *Shigella flexneri*, *Candida albicans* ATCC 35659 and *Candida albicans*. These germs come from the laboratory of bacteriology of the National Institute of Hygiene (INH) of Lomé except for *Candida albicans* ATCC 35659 which was provided by the Laboratory of Microbiology and Control of Foodstuffs (LAMICODA), ESTBA/UL. Clinical strains were isolated from urine (*E. coli*), pus (*S. aureus*, *P. aeruginosa*), stool (*S. typhi*, *S. flexneri*) and vaginal secretions (*C. albicans*) from patients (Figure 1).

2.1. Methods

2.1.1. Phytochemical Screening

Phytochemical compounds such as polyphenols, flavonoids, tannins, saponosides, terpenes, steroids, alkaloids, mucilages, coumarins, reducing compounds, anthocyanins, leucoanthocyanins, quinones and cyanogenic derivatives, free anthracene derivatives and glycosides have been sought in plant powder using methods described by Harbon [13] and Wong *et al.* [14].

2.1.2. Extraction

50 g of vegetable powder were macerated with 500 mL of ethanol 95° for 24 h with stirring at 1600 rpm. The residue was taken up twice in 300 mL of ethanol 95° before being rejected. For the hydroethanolic maceration the same technique was used with a water-ethanol mixture in equal volume (v/v). For the aqueous



Figure 1. Fruits of *Syzygium aromaticum*.

extract, a decoction was obtained from 50 g of vegetable powder boiled in 500 mL of distilled water for 30 min. The decoction obtained was filtered hot on cotton and then cooled at room temperature. Decoction and macerates were then filtered through Wattman N° 40 filter paper. Each filtrate was then evaporated under vacuum at 40°C using an evaporator. The three extracts were stored at -20°C until use.

2.1.3. Antimicrobial Activities

The germs collected were re-isolated on appropriate agar medium (Chapman for *S. aureus*, Eosin Blue Methylene (EMB) for *E. coli*, Hectoen for Salmonella and Shigella, Muller Hinton Agar (MHA) for Pseudomonas, Sabouraud chloramphenicol (SC) for *C. albicans* in order to obtain young colonies from 18 to 24 h which were used in the preparation of the inoculum. The tested extract solutions were prepared at a concentration of 200 mg/mL in distilled water and then sterilized on millipore membrane of 0.45 µm porosity and 47 mm in diameter.

1) Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed by the agar well diffusion method used by Karou *et al.* [15] with some changes. It is a presumptive test that has made it possible to identify the active extracts starting from a high concentration.

The microbial suspensions used were equal to 0.5 Mac Farland ($\approx 10^8$ CFU/mL). They were quantified by the measurement of densitometer turbidity. The inoculum was introduced on culture medium prepared under standard conditions. These were MHA agar for bacteria and Casitone agar for Candida. The quality of these medium was evaluated by sterility and fertility tests before use. After inoculation of the medium, wells of 6 mm in diameter were made using a sterile hollow punch concentrically in the agar. Each well was flooded with 50 µL of extract at a concentration 200 mg/mL. Gentamicin solutions 10 µg (for staphylococci, Enterobacteriaceae, and Pseudomonas) and Nystatin 100 µg for *C. albicans* were used as reference drugs. For negative controls, sterile distilled water was used in place of the extract. After 30 minutes of pre-diffusion at laboratory temperature, the Petri dishes were incubated for 18 to 24 h at a temperature of $35^\circ\text{C} \pm 2^\circ\text{C}$ for the bacteria and $25^\circ\text{C} \pm 2^\circ\text{C}$ for the yeasts. The diameters of the microbial growth inhibition zones were measured using an electronic reading chart. Extracts having an inhibition diameter ≥ 12 mm (including disc) were used for the determination of MIC, MBC/MFC. The tests were repeated in triplicate.

2) Determination of Minimum Inhibitory Concentrations (MIC) and Bactericidal/Fungicidal (MBC/MFC)

The test for establishing the susceptibility curve was carried out for the extracts which gave a growth inhibition diameter of the seeds ≥ 12 mm with the previously performed antibiotic susceptibility testing (presumptive test). It was performed using the 96-well microplate dilution method [16] [17] [18]. From a stock solution of 200 mg/mL extract, a series of successive dilutions by geometric

progression of reason 2 were made in Mueller Hinton Broth (MHB). The wells were inoculated with a microbial suspension at 6×10^5 CFU/mL. Quality control was done with MHB (not inoculated). Another control was made with seeded MHB to facilitate reading. The tests were carried out under a Microbiological Safety Post (PSM). The preparations were covered with parafilm and then incubated at the appropriate temperature for 24 h. After incubation, the wells were observed with the naked eye. The presence of turbidity or deposition corresponded to the presence of microbial culture. The well corresponding to the lowest concentration of extract for which no culture was observed represented the MIC of the extract on the strain tested. Then, from the MIC, 100 μ L were taken from wells that did not give microbial growth visible to the naked eye and then seeded on Plate Count Agar (PCA) medium for bacteria and SC agar for *Candida*. The incubation was carried out at the appropriate temperature for 24 h. The lowest concentration for which no colony was considered as the MBC or MFC of the extract on the strain tested. The antibiotic potency of the extract on the microbial strain was determined by the MBC/MIC ratio. The tests were performed in triplicate [15] [18].

2.1.4. Data Analysis

The results were analyzed using Graph Pad Prism 6 software and then presented as the mean with standard deviation (Mean \pm SD), $\alpha = 0.05$.

3. Results

3.1. Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids, gallic tannins, flavonoids, anthocyanins, saponosides, terpenes, steroids, coumarins, and reducing compounds. The catechoic tannins, the leucoanthocyanins, the quinones, the cyanogenic derivatives, the mucilages, the glycosides (free, O-, C- and cardiac) being absent (Table 1).

3.2. Presumptive Testing

The three extracts were active on all tested germs with growth inhibition diameters ≥ 12 mm except for the aqueous extract which gave a diameter of inhibition < 12 mm for salmonella and yeasts. In general, the growth inhibition diameters of the seeds range from 0 to 21 mm for the aqueous extract, from 12 to 28 mm for the hydroethanolic extract and from 0 to 26 mm for the ethanolic extract (Table 2 and Figure 2).

Table 1. Phytochemicals screening.

Metabolites	Al	Tac	Tag	Fl	Ant	Leu	Qn	Sp	Tp	St	Cy	Mu	Cm	Cr	Hl	O-H	C-H	Hc
<i>S. aromaticum</i>	+	-	+	+	+	-	-	+	+	+	-	-	+	+	-	-	-	-

+: Presence; -: Absence; Al: Alkaloids; Tac: Catechic Tannins; Tag: Gallic tannins; Fl: Flavonoids; Ant: Anthocyanins; Leu: Leucoanthocyanes; Qn: Quinones; Sp: Saponosides; Tp: Terpenes; St: Steroids; Cy: Cyanogenic derivatives; Mu: Mucilages; Cm: Coumarins; Cr: Reducing compounds; Hl: Free glycoside; O-H: O-Glycosides; C-H: C-Glycosides; Hc: Cardiac glycoside.

Table 2. Susceptibility of microorganisms to extracts.

Microorganisms	<i>S. aromaticum</i> extracts			Positive controls		Negative control
	Aqueous	HydroEtOH	H ₂ O	Gentamicin	Nystatin	H ₂ O
<i>Escherichia coli</i> ATCC 25922	16 ± 0	18 ± 0	12 ± 1	16 ± 0	NA	0 ± 0
<i>Escherichia coli</i>	15 ± 1	17 ± 0	14 ± 0	14 ± 0	NA	0 ± 0
<i>Staphylococcus aureus</i> ATCC 29213	19 ± 0	22 ± 0	26 ± 0	24 ± 0	NA	0 ± 0
<i>Staphylococcus aureus</i>	20 ± 0	21 ± 0	22 ± 0	17 ± 0	NA	0 ± 0
<i>Salmonella typhimurium</i> ATCC 14028	9 ± 0	12 ± 0	14 ± 0	16 ± 0	NA	0 ± 0
<i>Salmonella typhi</i>	0.00	17 ± 1	16 ± 0	18 ± 1	NA	0 ± 0
<i>Pseudomonas aeruginosa</i> ATCC 27853	13 ± 0	19 ± 1	14 ± 0	15 ± 0	NA	0 ± 0
<i>Pseudomonas aeruginosa</i>	15 ± 0	14 ± 0	14 ± 0	15 ± 0	NA	0 ± 0
<i>Shigella flexneri</i> ATCC 12022	21 ± 1	22 ± 0	14 ± 0	15 ± 0	NA	0 ± 0
<i>Shigella flexneri</i>	21 ± 0	22 ± 0	24 ± 0	15 ± 0	NA	0 ± 0
<i>Candida albicans</i> ATCC 35659	0 ± 0	27 ± 1	17 ± 0	NA	16 ± 1	0 ± 0
<i>Candida albicans</i>	0 ± 0	28 ± 0	21 ± 0	NA	17 ± 1	0 ± 0

HydroEtOH: Hydroethanolic; Et-OH: Ethanolic; NA: Not applicable.

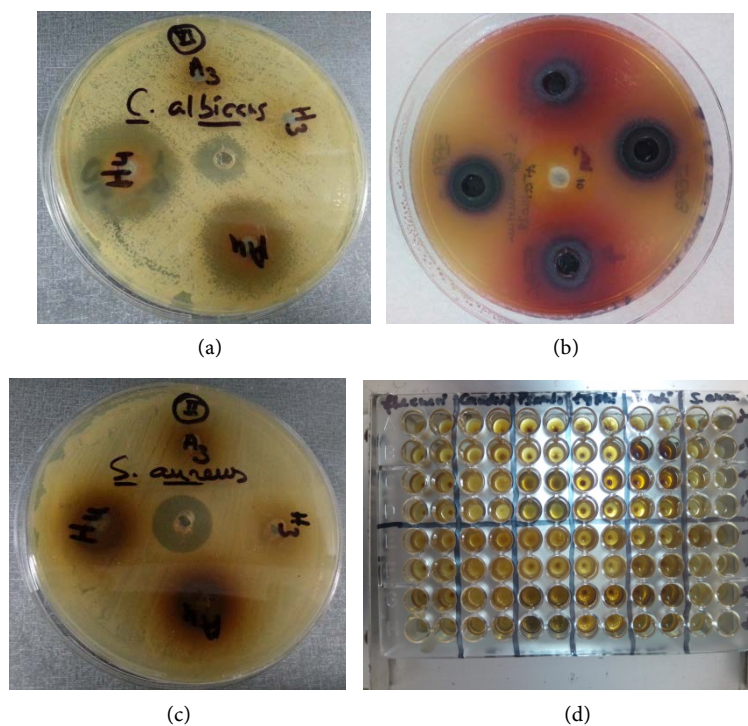


Figure 2. Typical images showing the inhibition zones. (a) Effects of hydroethanolic extract (H4) and aqueous extract (A4) against *C. albicans*; (b) Effect of ethanolic extract against *S. typhimurium*; (c) Effects of hydroethanolic extract (H4) and aqueous extract (A4) against *S. aureus*; (d) Determination of MIC in microplate.

3.3. Microbicidal Activity

MICs and MBCs/MFCs were determined for germs that were susceptible to ex-

tracts with inhibition diameters ≥ 12 mm. The bacteriostatic and bactericidal effects of the extracts on the germs were determined by the ratio of MBC/MIC or MFC/MIC ≤ 1 (Bactericidal); MBC/MIC or MFC/MIC ≥ 2 (Bacteriostatic). The results are compiled in **Table 3**.

MICs of the aqueous extract ranged from 0.0976 to 0.3906 mg/mL for both reference and clinical microorganisms with some differences for some strains. MBC and MFC are twice as high as MICs for both germ types; which gave a ratio of MBC/MIC or MFC/MIC = 2 for the aqueous extract. For the hydroethanolic extract, the MICs and MBCs or MFCs are between 0.0488 and 0.7812 mg/mL and the MBC/MIC or MFC/MIC ratio = 2 for most germs; only three germs (*E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 35659) had their MBC/MIC or MFC/MIC = 1. The microorganisms were also sensitive to the ethanolic extract with MIC and MBC/MFC between 0.0976 and 0.7812 mg/mL. Four germs showed a MBC/MIC or MFC/MIC = 1 ratio; this ratio is greater than or equal to 2 for the other germs and even 4 for *S. flexneri*. All clinical strains were inhibited with concentrations equal to or greater than those corresponding to inhibition of standard germs.

4. Discussion

The study of the antimicrobial activity of *S. aromaticum* made it possible to determine the sensitivity of *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. typhimurium* ATCC 14028, *P. aeruginosa* ATCC 27853, *S. flexneri* ATCC 12022, *C. albicans* ATCC 35659 and their clinical counterpart (*E. coli*, *S. aureus*, *S. typhi*, *P. aeruginosa*, *S. flexneri*, *C. albicans*) to aqueous, hydroethanolic and ethanolic extracts. The aqueous extract inhibited approximately 66.67% of tested germs

Table 3. MIC and MBC of microorganisms.

Reference germs	Aqueous			HydroEtOH			Et-OH		
	CMI	MBC/MFC (mg/mL)	MBC/CMI MFC/CMI	CMI	MBC/MFC (mg/mL)	MBC/CMI MFC/CMI	CMI	MBC/MFC (mg/mL)	MBC/CMI MFC/CMI
<i>Escherichia coli</i> ATCC 25922	0.0976	0.1953	2	0.0976	0.0976	1	0.0976	0.1953	2
<i>Escherichia coli</i>	0.0976	0.1953	2	0.0976	0.1953	2	0.0976	0.1953	2
<i>Staphylococcus aureus</i> ATCC 29213	0.0976	0.1953	2	0.0976	0.1953	2	0.0976	0.0976	1
<i>Staphylococcus aureus</i>	0.0976	0.1953	2	0.0976	0.1953	2	0.0976	0.1953	2
<i>Salmonella typhimurium</i> ATCC 14028	-	-	-	0.1953	0.1953	1	0.3906	0.7812	2
<i>Salmonella typhi</i>	-	-	-	0.1953	0.7812	2	0.3906	0.7812	2
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.3906	0.7812	2	0.3906	0.7812	2	0.3906	0.3906	1
<i>Pseudomonas aeruginosa</i>	0.3906	0.7812	2	0.3906	0.7812	2	0.7812	0.7812	1
<i>Shigella flexneri</i> ATCC 12022	0.0976	0.1953	2	0.0976	0.1953	2	0.0976	0.3906	4
<i>Shigella flexneri</i>	0.3906	0.7812	2	0.3906	0.7812	2	0.1953	0.3906	2
<i>Candida albicans</i> ATCC 35659	-	-	-	0.0488	0.0488	1	0.0976	0.0976	1
<i>Candida albicans</i>	-	-	-	0.0488	0.0976	2	0.0976	0.1953	2

while the hydroethanolic and ethanolic extract inhibited all the germs (100%). The germs were totally susceptible to the reference drugs.

E. coli ATCC 25922 and *E. coli* clinical strain were susceptible to aqueous, hydroethanolic and ethanolic extracts with inhibition diameters ranging from 12 to 18 mm. The largest diameters were found with the hydroethanolic extract; diameters that are greater than those given by Gentamicin used as a reference drug. The MICs and MBCs of the aqueous and ethanolic extracts on the reference strain and the clinical strain are identical, but with $MBC = 2MIC$; giving a ratio $MBC/MIC = 2$ and determining bacteriostatic activity of these extracts on the two strains of *E. coli*. As for the hydroethanolic extract, the MICs and MBCs are identical to the previous ones on the clinical strain, but on the standard strain, the MIC is equal to the MBC and consequently, $MBC/MIC = 1$ determining its bactericidal activity. Alharbi *et al.* [3] previously investigated the antimicrobial activity of *S. aromaticum*; but their study focused on the essential oils of the plant. According to their study, the pure oil of *S. aromaticum* inhibited the clinical strains of *E. coli* with MICs between 2.5 and 3.75 $\mu\text{g/mL}$ and MBCs between 5.0 and 7.5 $\mu\text{g/mL}$; giving a bacteriostatic activity. Jimoh *et al.* [19] found instead a bactericidal activity of essential oils of the plant with a growth inhibition diameter of around 28 mm and MICs and MBCs of 5 $\mu\text{L/mL}$. The essential oil of the plant also gave growth inhibition of *E. coli* ATCC 25922 up to 14 mm in diameter at a concentration of 25 mg/mL [20]. These data confirm the antibacterial activity of our three brut extracts (aqueous, hydroethanolic, ethanolic) which gave a bacteriostatic activity on the clinical and standard strains of *E. coli*.

S. aureus ATCC 29213 and *S. aureus* were also sensitive to aqueous, hydroethanolic and ethanolic extracts with growth inhibition diameters ranging from 19 to 26 mm. The aqueous extract gave the best growth inhibition diameters that are even higher than those of the standard drug (Gentamicin). With the three extracts, the $MBC = 2MBC$ on both *S. aureus* strains except for the aqueous extract which gave a $MBC = MIC$ on the reference strain. The aqueous extract therefore has a bactericidal activity on *S. aureus* ATCC 29213 and bacteriostatic on the clinical strain. The effect of the other two extracts is bacteriostatic on both strains. Other studies carried out on *S. aromaticum* have shown that the pure oil of the plant has a bacteriostatic activity on strains of *S. aureus* Meti-R with MICs and MBCs respectively of 0.625 - 1.25 $\mu\text{g/mL}$ and 1.25 - 2.50 $\mu\text{g/mL}$ [3]. Bactericidal activity of the essential oil of the plant was found by Jimoh *et al.* [19], which obtained an inhibition diameter of 30 mm and a MIC and MBC of 5 $\mu\text{L/mL}$ on the seed. Saikumari *et al.* [21] studied the essential oil and the methanolic extract of *S. aromaticum* which gave inhibition diameters of 14 mm and 16 mm respectively at the concentration of 100 $\mu\text{L/mL}$. These same activities were found with our extracts. According to the study of Mohamed and Badri [20], the essential oil of the plant inhibits the growth of *S. aureus* ATCC 25923 with an inhibition diameter of 16 mm at a concentration of 25 mg/mL.

The actions of the aqueous, hydroethanolic and ethanolic extracts were irregular on *S. typhimurium* ATCC 14028 and *S. typhi*. Inhibition diameters of the aqueous extract on both strains are less than 12 mm; for this purpose it is considered inactive. The active extracts (hydroethanolic and ethanolic) have a growth inhibition diameter of between 12 and 17 mm; these diameters are smaller than those of Gentamicin used as a reference drug. The MICs and MBCs of the ethanolic extract are identical and the $MBC = 2MIC$; by deduction, $MBC/MIC = 2$, thus showing a bacteriostatic activity. The hydroethanolic extract has a bacteriostatic activity on the clinical strain and a bactericidal activity on the standard strain. The hydroethanolic extract was active against the both strains. Mohamed and Badri [20], by studying the antimicrobial activity of the plant on the reference strains showed that the essential oil of *S. aromaticum* inhibits the growth of *S. typhi* ATCC 6539 with a diameter of 15 mm at the concentration 25 mg/mL. This highlights the antibacterial activity of *S. aromaticum* on Salmonella strains.

The effect of extracts on *P. aeruginosa* ATCC 27853 and its clinical counterpart *P. aeruginosa* was significant compared to that of Gentamicin with inhibition diameters between 13 and 19 mm. The ethanolic extract was more active with inhibition diameters greater than those of the reference drug. The MICs are the same except for the ethanol extract, which has a higher MIC on the clinical strain. The $MBC = 2MIC$ except for the ethanolic extract whose $MIC = MBC$ on the reference strain. It appears that the ethanolic extract has a bactericidal activity on *P. aeruginosa* ATCC 27853 and bacteriostatic on *P. aeruginosa* clinical strain. The aqueous and hydroethanolic extracts showed bacteriostatic activity on both clinical strains. The study of the antibacterial activity of pure oils of *S. aromaticum* on the clinical *P. aeruginosa* strains conducted by Alharbi *et al.* [3] proves the bacteriostatic and bactericidal effect of our brut extracts on *P. aeruginosa*. They showed that the pure oil of *S. aromaticum* inhibits *P. aeruginosa* with MICs and MBCs ranging from 0.3125 - 0.625 $\mu\text{g/mL}$ and 0.625 - 0.9375 $\mu\text{g/mL}$ respectively and giving the bacteriostatic activity of the oil. Jimoh *et al.* [19] also reported the bacteriostatic activity of the essential oil of the plant which inhibits the growth of *P. aeruginosa* with a diameter of 21 mm and a MIC of 9 $\mu\text{L/mL}$. The literature review also showed that the reference strain, *P. aeruginosa* ATCC 27853 was sensitive to the essential oil of *S. aromaticum* with a zone of growth inhibition equal to 16 mm in diameter at the concentration of 50 mg/mL [20], thus attesting the antibacterial activity of the plant on the *P. aeruginosa* standard strain.

Shigella were also responding to the aqueous, hydroethanolic and ethanolic extracts of the plant. *S. flexneri* ATCC 12022 and *S. flexneri* were sensitive to all three extracts with growth inhibition diameters ranging from 14 to 24 mm. Both strains of Shigella were also susceptible to Gentamicin. The extracts all gave a bacteriostatic activity on the two strains with a ratio $MBC/MIC = 2$ and 4 exceptionally for the ethanolic extract on the standard strain. In general, the hydroethanolic extract was more active.

The yeasts showed resistance to the aqueous extract. *C. albicans* ATCC 35659 and *C. albicans*, clinical strain was sensitive to hydroethanolic and ethanolic extracts with inhibition diameters between 17 and 28 mm. Both strains were also susceptible to Nystatin as a standard. The hydroethanolic and ethanolic extracts gave a fungicidal activity on the standard and fungistatic strain on the clinical strain. The MICs of the ethanolic extract on *Candida* were lower than those observed with bacteria. Other studies by Mohamed and Badri [20] revealed the antifungal activity of the essential oil of the plant on *C. albicans* ATCC 7596. The results of their studies showed that the yeast was susceptible with a diameter of inhibition of 20 mm at the concentration of 12.5 mg/mL. Jimoh *et al.* [19] showed that the essential oil of *S. aromaticum* has bacteriostatic activity on *C. albicans* with an inhibition diameter of up to 44 mm and a MIC of 1 μ L/mL. This confirms the antifungal activity of our hydroethanolic and ethanolic extracts.

Phytochemical compounds (alkaloids, tannins, flavonoids, anthocyanins, saponosides, terpenes, steroids, coumarins, reducing compounds) found in the fruits of *S. aromaticum* have been proven by some authors [19]. The antimicrobial activity observed with the aqueous extracts, hydroethanolic and ethanolic would be at the origin of one or more of these phytochemical compounds which would have acted alone or in synergy. Eugenol, a phytochemical compound mainly found in clove and already known for its antibacterial and antifungal activity, may be responsible for the observed antimicrobial activity [22] [23]. In fact, the alkaloids, saponosides, flavonoids and tannins in the fruits of the plant are known to have a curative activity against several pathogenic agents: *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* [19] [24]. The action mechanism of tannins is complexation either with enzymes or with bacterial substrates or with metal ions or its action on the cell membrane of bacteria [18] [25]. Tannins are known to have inhibitory activities on the growth of *S. aureus* and *P. aeruginosa* [26]. Flavonoids cause lysis of the membrane and consequently death of the cell [18] [27]. These mechanisms of action of tannins and flavonoids could partly explain the bactericidal and bacteriostatic effects given by the aqueous, hydroethanolic and ethanolic extracts of *S. aromaticum* on the tested microorganisms.

5. Conclusion

This study confirmed the antimicrobial activity of *S. aromaticum* fruits used in the treatment of infections by practitioners of traditional medicine. The sensitivity tests carried out on the reference strains and their clinical counterpart showed that the hydroethanolic extract was very active on all these tested germs and it would therefore be desirable to extend the tests to multidrug-resistant germs. The antimicrobial activity of aqueous extracts, hydroethanolic and ethanolic observed could be related to certain phytochemical compounds contained in the fruits of the plant. Furthermore, studies on the determination of the physicochemical constants, pharmacokinetics, pharmacodynamics and toxicity of

the extracts must be pursued in order to produce traditionally improved medicinal products to replace alcoholic herbal infusions of medicinal plants since the alcohol exerts toxicity on the organism.

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Conflicts of Interest

No conflict of interest.

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